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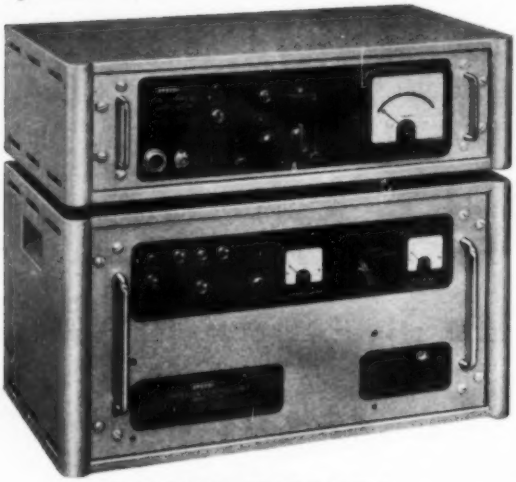

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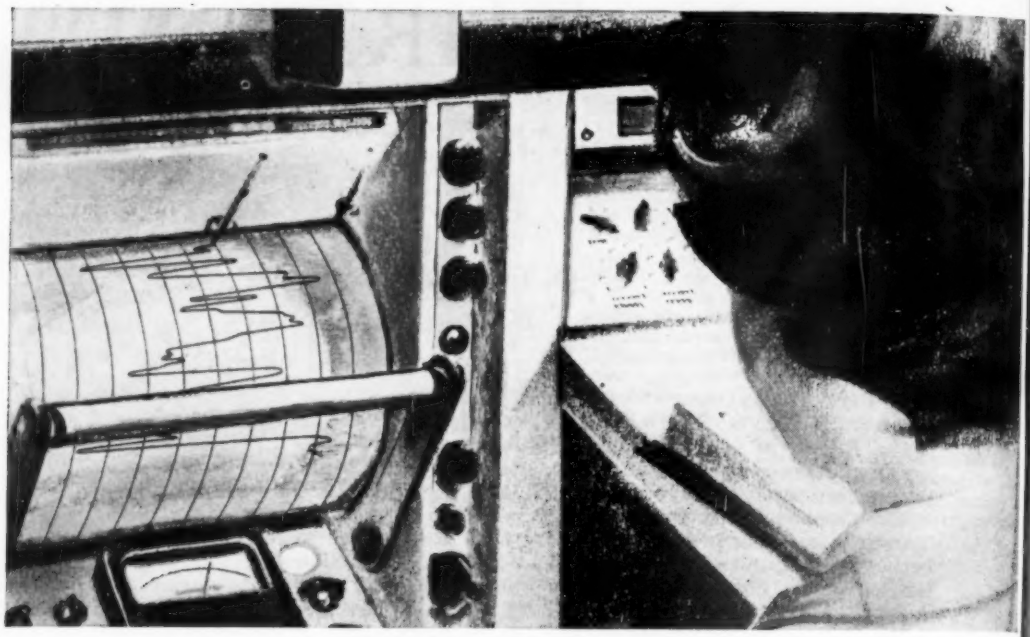

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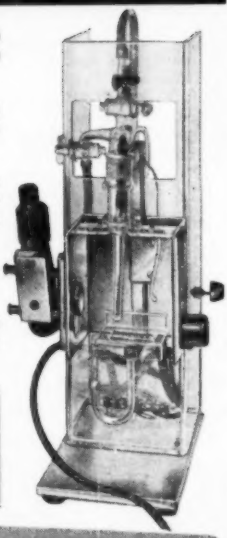

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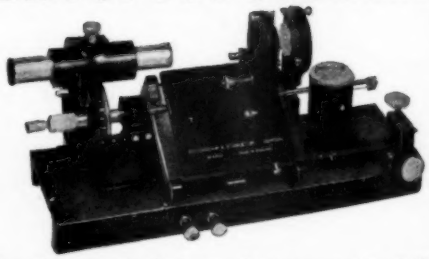

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PHLOGISTON OR ATOMS?

WE hear a great deal these days about the need for more scientific and technological education, both to produce more scientists and technologists and to give the layman some idea of what they are doing; but the method adopted for this form of education is comparatively neglected. It seems to be assumed by too many people that the present school courses are quite adequate and that it is simply a matter of pushing more people through them. A report* by Dr. Michael Bassey, on a survey he carried out recently on what some arts students at the University of London remembered and thought of the chemistry they had learned at school, strongly suggests that there is something seriously wrong with elementary school chemistry, as presented to the person who does not intend to do any more science in any event; and what is true of chemistry is probably true of the other sciences.

One of Dr. Bassey's questions was: "Which of air, water, clay and wood contains atoms?" One in five got the answer wrong. A few of them may have thought he was trying to find out if they knew the difference between elements, compounds and mixtures; even so, the proportion is far too high. All the people questioned had passed at least chemistry in general science of the General Certificate of Education (Ordinary Level) and they were all university students. Elementary chemistry obviously cannot cover a great deal of ground, yet surely it should manage to instil the basis of all chemistry, that is, the atomic theory.

Since all the people who replied to the questionnaire had passed some form of chemistry (Ordinary Level), their ignorance cannot be due simply to inattention. What, then, were they doing during the years they studied chemistry? Another of Dr. Bassey's questions provides the answer: Were you taught about aspirin, DDT, Lavoisier, phlogiston and the Law of Multiple Proportions? In Dr. Bassey's own words: "Lavoisier came out top of the poll, phlogiston and the Law of Multiple Proportions shared the second place, and DDT and aspirin would have lost their deposits in a parliamentary election". In fact, the teaching of elementary chemistry is apparently dominated by a historical approach which tends to exclude modern developments.

It can, of course, be argued that in the short time available the historical approach will give the best understanding of the subject. The phlogiston theory is an excellent example of a hypothesis that fitted most of the facts available at the time. Lavoisier's experiments are probably the most important series in the whole history of chemistry. The determination of the composition of water does illustrate the sort of approach that is used in all the sciences. But to grasp the significance of such things requires a great deal of background knowledge which the ordinary schoolboy is certain not to possess, and a feat of historical imagination of which he is most unlikely to be capable. If he does not grasp their significance he feels that they are irrelevant and dull. A youngster who knows the formula of water is H_2O is apt to be contemptuous of anyone who does not.

What should elementary chemistry, or for that matter elementary science, try to do? Obviously it has to provide some sort of grounding: the scientific equivalents of addition, subtraction, multiplication and division. It should also create interest, in the sense that even someone who only intends to do science for a few years to pass the necessary examinations should be able to see why others should want to devote most of their time to it. But it is essential that it should not create the hostility that comes from boredom, and bored most of the people who replied to Dr. Bassey's questionnaire certainly seemed to have been. Most of them put this down to chemistry, as taught to them, being a lot of unconnected, uninteresting facts, totally unrelated to everyday life.

This is not really surprising when one considers the present syllabus. Its attempts to include everyday and commercial applications look as though they were put in fifty years ago, which they probably were. Washing soda! Bleaching powder! An obsolete process for manufacturing sulphuric acid! Most of the important developments in chemistry in the past twenty years or so have come from the organic field—plastics, drugs, insecticides—yet organic chemistry does not even appear in the Ordinary Level syllabus. One needs a certain amount of basic knowledge before starting organic chemistry, of course, but there might at least be some hint of the fact that carbon forms long chains and rings and that these are the basis of life. The real trouble with the historical approach is not that it is there, but that it seems to displace everything else.

The criticism that chemistry is taught as an unconnected series of facts is all the more regrettable, since it can be taught in a way that makes it a coherent body of knowledge, developing logically from a few basic ideas, leading straight to many interesting and stimulating modern developments, and emphasizing the close connexion between chemistry and physics. The way is simply to start with the atom. The structure of the atom leads naturally to the periodic classification of the elements, and here some properties of the elements can be introduced not as isolated facts but to show how the elements are related to one another. Valency follows from atomic structure. Ionization, crystals, states of matter, can all be dealt with in terms of the atom. In fact, the whole subject can be brought together, and at each point items of everyday and topical interest can be introduced—the new metals, atomic energy, rocket fuels—things that stimulate interest instead of killing it.

* *New Scientist*, **7**, 1594 (1960).

The atomic theory can be expressed quite easily in simple terms; so there is really no justification for saying that it is too advanced to be understood by children in their early teens. After all, the study of electricity nearly always begins with electrostatic attraction. The force that attracts the electrons to the nucleus can be demonstrated with a fountain pen rubbed on the sleeve and a bit of paper. There is the analogy with the solar system, and atomic structure lends itself very well to demonstration by diagrams and models. It is certainly a good deal easier to understand the elementary theory of the atom than to grasp the significance of the phlogiston theory.

Dr. Bassey's questionnaire was sent to arts students; but these arguments apply equally well in the case of people who intend to specialize in science. The fact that they have an interest in science is no excuse for a boring syllabus. The defects in school chemistry are particularly obvious at the elementary stage, but they persist right through the school. Is there any real justification for the amount of time devoted to the extraction of metals at General Certificate of Education (Advanced Level), for example? A good deal of irrelevant material could probably be found in university courses as well.

The teachers cannot be blamed for this state of affairs, although most people are reluctant to change a well-established routine, since they have to prepare their pupils for examinations based on an out-of-date syllabus. But they are represented on the committees that decide what shall be included, so they do have an opportunity to try to improve things. Books, too, tend to be written around a syllabus. In fact, there are a lot of vested interests that will probably oppose any far-reaching changes, and far-reaching changes are certainly due.

Every year there is more and more to be learned; if the new things are not to be neglected, as they tend to be at present, a continuing review of examination syllabuses will be necessary, to prune off things that have lost their importance. If no pruning is done the syllabus will grow so fast that it will soon become quite unrealistic. The historical approach should not be dropped automatically; in many cases it may be very suitable, but the question, "What is the best approach?", should always be asked before any approach is adopted.

LAND USE AND THE CYPRUS PROBLEM

The Evolution of the Rural Land Use Pattern in Cyprus

By Dr. Demetrious Christodoulou. With Land Use Map, 1 : 253,440, prepared by R. R. Rawson and Dr. K. R. Sealy. (The World Land Use Survey—Regional Monograph No. 2.) Pp. vi+230. (Bude, Cornwall: Geographical Publications, Ltd.; London: Ed. Stanford, Ltd., 1959.) 25*s*.

FOLLOWING the success of Dr. Tregear's monograph on "Land Use in Hong Kong" (1958), the World Land Use Survey, under Prof. Dudley Stamp's direction, has produced a valuable, and topical, explanatory account of the land use problem in Cyprus. This publication contains what are, in effect, two separate though complementary studies married together by careful editing.

One of these studies, the "Land Utilisation Map of Cyprus", on a scale of approximately 1 : 250,000, was first published in 1956, but it is a great convenience to have it for reference alongside Dr. Christodoulou's monograph. The map itself was prepared from some 10,000 air photographs by a team of workers at the London School of Economics directed by Mr. R. R. Rawson and Dr. K. R. Sealy and records nine categories of use.

Dr. Christodoulou's study reflects, in its form of publication, the earlier publications of the Land Utilization of Britain; in its methods, the author's geographical training in Britain at Southampton and at the London School of Economics; and in the quality and spirit of the writing, his own long experience in the country of his birth, of which he is now land consolidation officer. More recently he has become chairman of a Food and Agriculture Organization working party on the problems of administration of public and common grazing lands in southern Europe, and readers of his study of Cyprus will await his report with more than usual interest.

Though the third largest island in the Mediterranean, Cyprus is no bigger than two English counties, such as Norfolk and Suffolk. The land, and its water supplies, remain the main natural resources, largely supporting a population of 550,000, now increasing at a rate of 1·5 per cent per annum. Marked regional contrasts exist in the degree to which population presses upon land, but overall the mounting population pressure, coupled with an increasing expectation of improved standards of living, are vital factors in the island's social, economic and political life. On the whole, society remains close to the land: about half the adult population is engaged primarily in farming.

The land-use pattern is a varied and complex one. Cyprus presents an unusual variety of cultivations and crops, with rapid variations occurring within a small compass. The complex situation is analysed carefully by a systematic treatment of the principal crop and land-use distributions, by a discussion of the main farming types and through a synthetic examination, region by region, of land use characteristics.

Dr. Christodoulou's assessment of the physical conditions affecting crop production, based upon a nine-fold regional division of land-forms, is straightforward and generally clear. Climate is given special attention in view of the importance of water to the life of this thirsty island. The fundamental significance of winter rainfall, and the adjustment to it of the life-cycle of vegetation and the pattern of human endeavour, are forcefully driven home. The sad record of recurring droughts arising from rainfall variability is long, and a critical treatment is given of schemes to conserve and utilize "the most precious resource" of the island.

But the diverse pattern of village and farm bears also the ineradicable marks of centuries of human labour. Christodoulou shows that, in the final analysis, it is the human or institutional problems, rather than the physical conditions, that provide the most stubborn problems of land use. His chapters on these problems form the most interesting

and original parts of the book, for in them Dr. Christodoulou's own personal feeling for the land and its people is most clearly revealed.

The gap between available scientific knowledge and the archaic practices of the mass of peasant farmers is seen to be at the core of the problem: technical education has scarcely begun to narrow the gap while, so long as agriculture remains the least remunerative occupation, there is little prospect of its attracting technically efficient and receptive farmers. Farming problems of to-day bear the burden of a complicated situation of land tenure, the antecedents of which are, in summary, unravelled. Christodoulou feels that much of "the malaise of the rural areas and a serious obstacle to modern agricultural techniques stem from the size and fragmentation of peasant holdings". Holdings are fragmented, plots dispersed and the pattern of ownership diffuse. The financial misfortunes of the Cyprus peasant are not overlooked, and, even if times are better than they were, capital is scarce, returns small and rural indebtedness is a continuing source of rural debilitation. Unfortunately, the section on the internal market is short, necessarily so in the absence of detailed studies, and the impact of town growth on the countryside, though many times suggested, is not separately considered. But there is an excellent account of the evolution and characteristics of the farm and of the main types of farm dwelling.

Dr. Christodoulou's work is critical and firmly based both on field work and on relevant British, Greek and Turkish sources. He is understandably cautious about the future. While not neglecting the many valuable contributions already made, he clearly feels that reorganization of the whole agrarian pattern is essential and that careful planning, based on a full appraisal of the human and natural resources of the island, is overdue.

Problems of land use are fundamental to the social, economic and political issues that beset this troubled island. It is a sad commentary that it is only in the closing days of British administration that such a study, and this from a private source, should become available. Christodoulou's work should quicken attention not only to these issues in Cyprus but also to the study of the use of land in other territories for which responsibility still lies in Britain.

M. J. WISE

SUBMARINE GEOLOGY

The Earth beneath the Sea

By Prof. Francis P. Shepard. Pp. xii+275. (Baltimore, Md.: The Johns Hopkins Press, 1959.) 5 dollars.

PROF. SHEPARD has made a lifelong study of submarine geology, but it is a rapidly advancing subject and he says he has obtained most of his education since he received his doctor's degree. He published his last book on submarine geology twelve years ago. It did not mention turbidity currents and now most students are convinced that they really do charge down continental slopes at the speed of express trains and carry silt hundreds of miles across the ocean floor. The book has much else that is new, largely in physical and geophysical treatment but also in topography. It is written for readers with little technical background though an elementary knowledge of geology or physical geography would be a help.

The author shows how every bit of the ocean floor from the shore lines to the greatest depth is likely to be influenced by currents, tides or some kind of oscillation, and deals successively with the beaches, continental shelves, slopes and the deep ocean floor. He maintains that the action of a lowered sea-level during glacial times was mainly responsible for the shelves, and that the canyons were cut at such times, or during periods of coastal elevation, and worked nowadays by turbidity currents. He is rather sceptical whether the trench of the mid-Atlantic ridge is as long and continuous a feature as recently claimed. In explaining flat-topped seamounts he favours the subsidence hypothesis, and that coral reefs occur where favourable growth conditions allowed the coral to keep pace with subsidence. He deals briefly with investigations using acoustic, seismic, gravity and heat-flow techniques and feels that the work is in too early a stage to allow much more than speculation about events below the ocean bottom.

When dealing with ripple marks, grading of sediments and other characteristics that have been used to date the past, he shows how many ideas have to be changed now that actual observations of what conditions are like on the sea bed have been made, and notes that we have been too much influenced by arguments that were largely philosophical.

G. E. R. DEACON

MINOR ELEMENTS IN SOILS

The Geochemistry of Rare and Dispersed Chemical Elements in Soils

By A. P. Vinogradov. Second edition, revised and enlarged. Translated from the Russian. Pp. ii+209. (New York: Consultants Bureau, Inc.; London: Chapman and Hall, Ltd., 1959.) 9.50 dollars; 80*s*. net.

THE behaviour of minor elements during the processes of rock weathering and soil formation is described and illustrated in A. P. Vinogradov's "The Geochemistry of Rare and Dispersed Chemical Elements in Soils", now in its second edition. The author assumes a sound knowledge of geochemistry and pedology, thereby limiting the use of the book to geochemists and soil scientists.

Chapter 3 on "General Geochemical Regularities" gives an outline of the various factors which control the distribution of elements in the zone of weathering. The following are briefly discussed: ionic radii, electromotive series, sorptive properties, ionic potential, *p*H, oxidation-reduction potentials, etc.

The major part of the book describes the distribution of the elements boron, fluorine, bromine, iodine, arsenic, selenium, lithium, rubidium, cæsium, strontium, barium, rare earths, titanium, zirconium, vanadium, chromium, manganese, nickel, cobalt, copper, zinc, cadmium, lead and molybdenum in soil profiles which are developed over the sedimentary rocks of the eastern European plain. The soil types dealt with are tundra, podzol, grey forest, chernozem, chestnut, desert serozem, brown soils, and red earths. Comparisons are frequently made with data from similar profiles described in non-Russian literature.

A few generalizations concerning the element under discussion are made at the beginning of each chapter. These are followed by a description of its content in profiles from the various soil-types. Finally,

conclusions of the behaviour of the element within the soil are given. Frequently these conclusions are not supported by the data quoted; for example, in Chapter 6 it is stated "in the majority of cases a tendency toward an increase in the content of fluorine in the subsoil was noted". However, in only two of the nineteen profiles presented (Table 20) is there an increase of fluorine in the subsoil. In the remaining seventeen examples quoted there is a decrease.

The behaviour of the elements tin, tungsten, radium, uranium, thorium, ionium, beryllium, scandium, gallium, indium, thallium, germanium, niobium, tantalum, antimony, bismuth, mercury, rhenium, gold, silver, platinum, tellurium and hafnium is briefly mentioned without introducing new data. A comprehensive bibliography contains 560 references, including many non-Russian papers.

Poor editing in the original Russian edition of the book is reflected in the English translation. Numerous errors appear in both the text and tables. Data have not been uniformly presented in some tables and often the units used are not clearly stated. The text on p. 170 should follow that on p. 167.

Despite the many editing errors, the extensive tables should prove useful sources of information on the normal contents of the minor elements in soils of the zonal types considered. A. H. Debnam

NUCLEAR RESEARCH IN THE U.S.S.R.

Atomic Energy in the Soviet Union

By Arnold Kramish. Pp. x+232. (Stanford, Calif.: Stanford University Press; London: Oxford University Press, 1960.) 27*s*. 6*d*. net.

THIS book by Arnold Kramish performs a most useful task in a thoroughly interesting fashion. The writer traces the published evidence of Soviet activity in the field of nuclear studies since before the War, and indeed he pays rather special attention to the important time of development in the early part of the 1930–40 decade. With some hesitation in the mid-war years only, he is able to establish a consistency and breadth of Soviet effort in the field that is altogether impressive. Many scientists who have neglected until recently the original Soviet publications will find the volume a considerable help in tracing some of the important landmarks in Soviet achievement in this field during the past thirty years.

During this time, as the author demonstrates, there have been many vagaries in the policy of the Soviet Union regarding the extent to which publicity has been given to particular advances. The book removes much of this difficulty, and the picture that emerges is one of almost uninterrupted pressure on the large number of well-trained scientists and engineers to make full use of the generous provision of support in both money and materials. As the author points out, our picture is still far from complete because of the secrecy that still surrounds many of the ventures. He points to interesting differences in approach to certain topics for example to the subject of controlled thermonuclear research. It appears to be the policy to be frank in those areas where really fundamental difficulties are present and so to encourage help to come from any possible source. It is no doubt rewarding to ponder on some of these basic differences in approach.

If one has to make any general conclusion from the study it is probably that achievement by scientists and engineers in the Soviet Union, the United States, and Great Britain is more or less proportional to the support afforded and relatively independent of the political background, provided that original thinking or inventiveness is encouraged.

The style of the author is lively and fresh, and the book is very easily digested by those with some background knowledge of modern physics and atomic energy. Its value to scientists, engineers and administrators is obvious, and it can be highly commended. The price is relatively modest at 27*s*. 6*d*., and the volume does not suffer from the absence of illustrations. S. C. Curran

PHYSIOLOGY OF MARINE ANIMALS

The Biology of Marine Animals

By J. A. Colin Nicol. Pp. xi + 707. (London: Sir Isaac Pitman and Sons, Ltd., 1960.) 95*s*. net.

TO those, of whom I am one, who for the past generation and more have worked in the field of comparative physiology of marine animals, this book comes as a revelation of the extent of achievement during this period. Very few of the 1,700 odd references to literature which are among the many virtues of this volume deal with papers earlier than the mid-twenties. It was in that earlier post-war period that the attention of marine biologists began to turn from description, of structure or of life-history, to analysis of function and to the elucidation of physiological as well as of morphological adaptation. Increased knowledge of marine environments went hand in hand with greater understanding of the internal fluid medium in animals and of how problems of osmoregulation had been solved. Interest extended from the properties of vertebrate hæmoglobin to those of hæmocyanin and of other invertebrate respiratory pigments, as well as to the wider general problems of animal pigments so wonderfully displayed in many marine animals. Then also knowledge of the complexities of the mechanisms of sensation and response began to include analysis of the sense organs and behaviour of marine invertebrates and fishes as well as of the nature of nervous and hormonal control particularly in fishes, cephalopods and crustaceans.

In this impressive volume of 700 pages, Dr. Nicol summarizes the results of these investigations, to which he has himself made very significant contributions. It is a major work of synthesis; it may well be the last time that anyone will attempt single-handed to cover so wide a field, in any event with that meticulous reference to the sources of knowledge here provided. It does not seem that this book could have been done better. It has been, one knew, years in preparation by an author living in the very atmosphere of marine biological research. It would appear, judging from the date of the introduction, to have been four years in process of publication. So, like all such books, it is a little out of date, a matter, however, that will only be apparent to active research workers and then only within their speciality.

Exception might be taken to the title, but then few people would agree about what precisely is meant by biology. Here it covers physiology, extending from body fluids to luminescence (on which the author writes with particular authority), but with final chapters on "Associations: Commensalism, Para-

sitism and Symbiosis" and "Skeletons, Shelters and Special Defences". There is no mention—and could not be without extension into a second volume—of reproduction, which one feels should be covered in any book dealing with biology. Dr. Nicol might have been better advised to restrict himself to his purely physiological chapters—with suitable change in title—because the final two chapters covering much wider, less-analysed fields are inevitably more condensed and less critical.

These, however, are very minor criticisms of a book that brings many into Dr. Nicol's debt. It is extremely well produced, containing some 300 figures as well chosen as they are reproduced. The publishers may well share some of the congratulations extended to the author but not, surely, when it comes to price. Was it, one may reasonably ask, really necessary to charge almost £5 for a book that should find a place in the library of every university and marine station in the world and be in the possession of everyone scientifically interested in marine animals?

C. M. YONGE

A NEW STUDY OF THE BRITISH ODONATA

Dragonflies

By Philip S. Corbet, Cynthia Longfield and N. W. Moore. (The New Naturalist: a Survey of British Natural History.) Pp. xii+260+32 plates. (London: William Collins, Sons and Co., Ltd., 1960.) 42*s*. net.

WITH just over forty species of dragonflies recorded from the British Isles, one might at first question the necessity for another book on these insects, since for the purposes of identification the subject has already been dealt with in two recent publications. The present volume in the "New Naturalist" series is, however, intended more for the naturalist than for the collector and may be considered rather as supplementary to Tillyard's classic, "The Biology of Dragonflies," in which the external and internal morphology of the Odonata was dealt with in great detail, but classification, distribution, fossil history, bionomics, etc., only occupied about one-third of the book. As in other volumes of the "New Naturalist" series, this book is an indication of the changed attitude of the naturalist, with the emphasis on the living insect and its behaviour rather than on dissection and museum study.

Three of its fourteen chapters deal with the composition of the British dragonfly fauna, its history and distribution, and four are devoted to the life-history of the various stages. The behaviour of the adult dragonflies, dispersal and seasonal regulation in dragonflies are the titles of other chapters, and the fossil history of the order and the inter-relationships of dragonflies and other animals (including man) are discussed. Various appendixes include a key to the larvæ of the British dragonflies, a survey of the wing venation and its terminology, methods of preserving colours of dragonflies and of marking dragonflies for behaviour studies.

The three authors are all general naturalists as well as being specialists in different facets of the study of Odonata. Dr. P. S. Corbet made a three-year investigation into various aspects of the life-history of dragonflies the subject of a thesis for his Ph.D. degree. Miss C. Longfield worked for thirty years as an honorary associate in the Department of Entomology, British Museum (Natural History), where she identified and described dragonflies from many parts of the world, including material collected on her own expeditions abroad. Dr. N. W. Moore made his investigations into the ecology and behaviour of adult dragonflies the basis of his Ph.D. degree, and has also studied Odonata in the field in Gambia.

These three authors, each being responsible for different sections of the book, have succeeded in welding their contributions into a homogeneous and very readable whole, without suppressing too much of their individual styles and personalities. Much of the information in the chapters on life-history and behaviour is based on the authors' personal studies, and the occasional use of the first person singular gives life to their accounts and prevents them from appearing too formal to the general reader. The inclusion (as an appendix) of Mr. A. E. Gardner's keys to the larvæ of British dragonflies, with his own illustrations, is a valuable addition to the book. These keys originally appeared in an entomological journal, and their reprinting here will introduce them to a wider public.

The production is entirely up to the standard of the previous volumes, and the work is fully illustrated with plates in both colour and in monochrome and with some line drawings. The line drawings in the chapter on behaviour are an excellent example of how to use bold, simple drawings. There are many colour plates, most of them reproduced from very fine transparencies specially taken by Mr. S. Beaufoy. These were mostly taken under controlled artificial light conditions and are in a sense studio portraits of the living insects. By this means a more uniform colour balance has been obtained, and the background can be chosen to suit the subject. All these colour plates are reproduced at natural size, and while this is an advantage for comparison, it does mean that the smaller specimens lose a certain amount of detail in reproduction. This is particularly noticeable where, as in one or two illustrations in the review copy, one of the colour impressions is slightly out of register. One realizes that to have reproduced the smaller species on a larger scale would have entailed more colour plates and an increase in cost.

As a whole, this is a most interesting book and one which should prove extremely useful not only to students of dragonflies but also to field naturalists and to students, as it not only gives a recent picture of the state of our knowledge of the British Odonata but also shows in what directions further research is needed.

D. E. KIMMINS

GROWTH AND FORM IN AN INSECT

The Control of Growth and Form

A Study of the Epidermal Cell in an Insect. By Prof. V. B. Wigglesworth. Pp. vi+140. (Ithaca, New York: Cornell University Press; London: Oxford University Press, 1959.) 21*s*. net.

THIS well-illustrated small book, based on six Messenger Lectures which Prof. V. B. Wigglesworth delivered to a general audience at Cornell University in 1958, displays the epidermal cell of an insect as a medium convenient for the

study of the principles of the control of growth and form. The author points out that this limitation of the field of study has both advantages and disadvantages, but the fact that the insect selected is *Rhodnius prolixus*, of which Prof. Wigglesworth has such deep and extensive knowledge, does much to outweigh the disadvantages.

Taking the view that there is much to be said for starting with specialized parts of a complex animal, rather than with a "simple" animal, Prof. Wigglesworth gives a brief description of the complex activities of the epidermal cells in producing the cuticle and later hardening it. He goes on to consider the special cells which become differentiated from the epidermis in the larva of *Rhodnius* and give rise either to dermal glands or sensilla, and puts forward the attractive hypothesis that the fate of the special cells is determined by the concentration of some essential chemical constituent in the epidermis. An epidermal cell which receives an appropriate amount of the essential factor develops into a sensillum, and one receiving a smaller amount into a dermal gland. The active cell first acquiring an effective concentration of the essential factor becomes dominant and by withdrawing the essential factor from its neighbourhood inhibits the development of other sensilla around it. Dermal glands may appear, however, suggesting that they need a lower concentration of the essential factor for their development, a view supported by their closer spacing.

Emphasizing that it is the capacity of the epidermal cells to produce cuticles of different types at different stages of the life-cycle which forms the basis of metamorphosis, Prof. Wigglesworth then discusses the hormones which initiate cuticle development and control its form. The epidermal cell possesses dual potentialities, and produces larval or adult cuticle according to its hormonal environment. It is stimulated to produce cuticle by the growth hormone, which is envisaged as controlling the synthesizing power of the cell by regulating permeability or transport. The juvenile hormone inhibits differentiation towards the adult by accelerating the production of the cuticle, and, since growth ceases when cuticle secretion begins, so limiting the period during which the epidermis can grow and produce a changed body form. Only in the absence of the juvenile hormone can the epidermal cell realize its latent potentiality of laying down cuticle of adult form. Metamorphosis and polymorphism are then discussed in terms of hormonal balance.

The final chapter deals with the integration of growth. The idea that tissue and body fluids convey chemical information on the activity of cells and tissues, so that the activity of one cell is reflected in an environmental change which regulates the activity of another cell, leads to the suggestion that the means of integration are present in generalized form in the epidermal cell and are not restricted to the specialized tissues involved in nervous conduction and hormone secretion.

The labours of many authors over the past twenty years and more have given us much information on the cuticle of insects, and caused us to have, like Prof. Wigglesworth, a profound respect for the epidermal cell which produces such a complex structure. It is refreshing now to have the cell itself considered against the wider background of the control of growth and form. Not only insect physiologists but also biologists generally will be grateful to Prof. Wigglesworth for this book.

R. Dennell

INTERNATIONAL CYTOLOGY

International Review of Cytology

Vol. 8. Edited by G. H. Bourne and J. F. Danielli. Pp. x+541. (New York: Academic Press, Inc. London: Academic Press, Inc. (London), Ltd., 1959.) 13 dollars.

THIS volume contains the useful address given to a plenary session of the International Congress for Cell Biology in September, 1957, by the veteran French cytologist, Charles Oberling, who has recently died. Besides Oberling on the structure of the cytoplasm, there are fourteen other contributors. Teachers of general zoology will be interested in the papers by Milton Fingerman (physiology of chromatophores) and C. F. Ehret and E. L. Powers (cell surface of *Paramecium*). Cell physiologists will note the work of D. B. Roodyn (metabolic studies on isolated nuclei), D. A. T. Dick (osmotic properties of living cells), H. Holter (pinocytosis) and I. M. Glynn (sodium and potassium movements in nerve muscle and red cells). Histologists will be helped by reading the papers by Leah Lowenstein (mammalian reticulocyte), David Hall (fibrous components of connective tissue, with special reference to elastic fibre), J. B. Bridges (experimental heterotopic ossification) and E. de Robertis (submicroscopic morphology of the synapse). In addition R. D. Preston has considered the wall organization in plant cells, and Bert Vallee and Frederic Hoch have a paper on trace elements in cellular function. The various authors come from France, the United States, Denmark, Argentina, Great Britain and Ireland. It is, of course, an honour to have a paper accepted for this splendid series, and the present contributions are correspondingly valuable.

Some of the papers, such as those of Roodyn, Dick and Glynn, are very complicated and hard going. Where possible the authors have discussed the electron microscopy of their field. The work of Ehret and Powers, which recalls that of Sedar, Porter, Manton and Roth, reminds us that morphological protistology is being re-written by electron microscopists. In his essay, Oberling touched on the past and looked to the future. He mentioned the dismissal in past times as artefacts of all granular, reticular and spongy structures in the cytoplasm, which had led to so much controversy. He wrote: "My generation has lived through this period, and those who in spite of their disappointments have remained morphologists, suffered a good deal". He attributes the past confusion to the physicochemists. This is only partly true. Up to very recent times the cytologists themselves provided most of the ground for controversy. In 1927–28 Covell and Scott stated that the Golgi apparatus of neurones was formed of neutral red-staining droplets, a view supported by E. V. Cowdry in his widely used "Text Book of Histology" (1938); and in 1949, Palade and Claude considered that the Golgi apparatus was formed of myelin droplets, which were supposed to be something different from the germ cell dictyosome (Golgi body). These papers emanated from the Rockefeller Laboratory, and the basic views they advocated were unsound. It is scarcely necessary in Britain to recall the confusion of views at the Royal Microscopical Society's symposium on the Golgi apparatus in 1955.

The position to-day is that, leaving aside ergastoplasm (and plastids), there are three recognized and separate 'large' extra-nuclear cell inclusions—Golgi

apparatus, mitochondria and centriole. The latter is a short tube with nine spaced fibrils embedded in its wall. The mitochondria consist of a matrix surrounded by a double membrane, the inner infolded at intervals. The reasons for, and function of, these two membranes are unknown. The mitochondria can divide by binary or multiple fission (*en rosace*). The Golgi apparatus, which also can divide and grow, consists of a dictyosome or aggregated group of dictyosomes formed of layers of oval or rectangular collapsed saccules or membranes, piled up usually closely to form a polarized lozenge-shaped body, in the hollowed base of which a secretion globule may appear. Watery vacuoles, either peripheral or basal or both, almost always become associated with the membranes, and chains of such covered vacuoles constitute the canals of Cajal of neurones. So universal is the dictyosome in its similarity that only a specialist could express an opinion as to whether a given electron micrograph of a Golgi body belonged to *Nemobius*, *Homo*, *Chromulina* or *Elodea*. Here is a universal cell device (or apparatus), vaguely considered as a filter or secretory body, the manner of functioning of which also is unknown. The understanding of this universally similar morphology of the Golgi apparatus is one of the two great basic discoveries due to electron microscopy. The other basic discovery is the understanding of the universality of the 9+1 fibrils in cilia and flagella, a truly astonishing thing.

As to the future, Charles Oberling wrote: "Each degree of magnification needs its mode of preservation (of the cells), and I am afraid that it is this prerequisite and not the magnifying power of electron or proton microscopy which will prevent us for the time being, from filling the gap between morphology and chemistry. But let us enjoy the tremendous opportunities within our reach of rich harvests and exciting discoveries for generations of workers".

J. Brontë Gatenby

MEDICINE AS A SOCIAL SCIENCE

Mirage of Health

Utopias, Progress and Biological Change. By Dr. René Dubos. (World Perspectives, Vol. 18.) Pp. 221. (London: George Allen and Unwin, Ltd., 1960.) 21*s*. net.

THE increasing use of insecticides with their striking and sometimes frightening effects, our recent experience of myxomatosis as well as other odd changes in some bird and animal populations, have made most of us aware of the importance of 'animal' ecology. However, the notion of a 'human' ecology —a study of the balance between environment and human populations—is much less widespread. But the subject is certainly no less complex, and it may be a good deal more exciting if only because the so-called natural balance of things has for ages been knocked about by the whims and fancies of man. His growing ability to control his own destiny and the implications of this control are the broad subject of this book.

One of the whims and fancies which man has cherished down the ages in all societies is that perfect health and happiness are just around the corner. But a life free from pathogens, like the two-car Utopia for which we strive, has its snags, and is probably undesirable as well as impractical. It seems scarcely necessary to say that we know very little about even our major diseases. For example, all infections do not mean clinical illness; some of us with severely damaged arteries do not die of coronary occlusion, and disorganized and cancerous cells have an inexplicable way of disappearing from some patients. Illustrations such as these merely serve to underline the complexity of the interrelated causes of disease—the ecological system in which we live. They provide additional reasons, if any were needed, for throwing overboard the cosy doctrine of specific ætiology and prompt us yet again to reflect upon the wider repercussions of our attack upon disease.

That we live within these finely balanced systems has been recognized for many years and by many writers—its importance for cognitive psychology was emphasized by the *Gestalt* theorists of the 'thirties and for social medicine by Ryle and others in the late 'forties. René Dubos in 1960 has brought together a mass of historical, sociological and biological facts which constitute a fascinating and popular account of this human ecology, an account which is both exciting and provocative.

The World Perspective Series seeks "to reveal basic new trends in modern civilization, . . . and to point to the new consciousness which can contribute to a deeper understanding of the interrelation of man and the universe, the individual and society, and of the values shared by all people". These are high hopes and must be difficult of attainment. Dr. Dubos does not attempt anything so spectacular, but in a quiet way he reaches at least one of these ill-phrased objectives. He traces first the notions of an earthly paradise—of health and happiness—through historical times to the present day and shows how we have developed a touching and altogether unwarranted faith in laboratory science alone. That man can become completely healthy by the simple process of spending money on research is believed by most Americans and many Europeans. 'Health is purchasable' say the doctors, and the National Institutes of Health are embarrassed by the amount of research money which they control. Dr. Dubos emphasizes the contrast between these simple half-truths and the reluctance of Western people to alter ways of life along the lines advocated by the very research workers whose studies they have sponsored. As a result, the patterns change and there is less typhoid and cholera, but at the same time mental disease and heart disease, to say nothing of motor-vehicle accidents, are already taking their places as the epidemics of the future.

After describing biological and social adaptation in various species and communities, the author is in a position to guess at some of the 'risks' which might attend perfect health. Among these risks he sees a decline in our ability to resist biological stress. In short, he feels that were man ever to achieve 'healthiness', he might well be like so many laboratory animals, in danger of the etiolation which comes from an unchallenged existence.

The three related themes of danger—from the decline in the importance of biological selection, from the rising power of man to control his own destiny, and from the changing patterns of disease—thread fascinatingly through the rest of the book. The Plague, Darwin, Pasteur, Dr. Snow and cholera, the Irish famine, the concentration camps in Germany and the contributions of Greek medicine, are all used

to build a picture of man's health in relation to his changing social and biological environment. Dr. Dubos ends with a summary word of caution: the creation of a self-chosen pattern of life remains a gamble; Nature will strike back. "It is," as he says, "always risky to tamper with the natural balance of forces in nature."

One might reply: "Of course it is, but that is how we learn". But Dr. Dubos' book is much more than a plea for caution in our dangerously slap-dash thinking about health. It is a learned, well-written and delightful introduction to medicine as a social science. It must be read particularly by the new generation of doctors, the human biologists as yet unencumbered by the pile of facts and loose thinking that is curative medicine to-day.

E. Maurice Backett

SOME ASPECTS OF ATHEROSCLEROSIS

Hormones and Atherosclerosis

Proceedings of the Conference held in Brighton, Utah, March 11–14, 1958. Edited by Gregory Pincus. Pp. xvi+484. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1959.) 13.50 dollars.

Connective Tissue, Thrombosis, and Atherosclerosis

Proceedings of a Conference held at Princeton, N.J., May 12–14, 1958. Edited by Irvine H. Page. Pp. x+316. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1959.) 9.50 dollars.

THE proceedings of these Conferences are complementary to one another and contain a very worthwhile cross-section of much experimental work in the field of atherosclerosis.

"Hormones and Atherosclerosis" has a collection of 32 papers devoted to various endocrine influences in cholesterol metabolism in experimental animals and in humans. The book opens with short but excellent articles on the biosynthesis of cholesterol by Bloch and Popjak and these are followed by an authoritative account of cholesterol degradation to bile acids by Bergström. The subsequent essays are mainly devoted to the influences of various hormones on lipid metabolism in different species. In the early part of the book the emphasis is on the effects of the thyroid hormones on cholesterol biosynthesis, turnover and concentration in different tissues, by Gould, Milch, Boyd, Pick and others. A considerable part of the monograph is devoted to further experiments on the well-known effects of œstrogens on lipid metabolism, including a discussion on the prediction of œstrogenic side-effects of steroids in man by Drill and co-workers, and a series of essays on the possible usefulness of œstrogens as cholesterol depressants in man by Oliver, Katz, Robinson and Marmorston. The influences of the adrenal cortex and stress are dealt with by Friedman, Seifter, Rosenman, Adlersberg and Furman.

"Connective Tissue, Thrombosis, and Atherosclerosis" contains 16 articles covering a fairly wide spectrum of the field. Duguid presents a clear account of his concepts of atherogenesis and the role of connective tissues in arterial disease, while the chemistry of connective tissue receives a brief but stimulating treatment by Jackson.

The role of elastic tissue in atherosclerosis is briefly dealt with by Lansing, while an outline of the chemistry of the ground substance is treated by Meyer and his colleagues. Some of the metabolic activities of fibroblasts as studied by the implanted sponge method are reviewed by Noble and her associates.

Blood coagulation, fibrinolysis and the countless ramifications of this complex field are treated by Ratnoff, Astrup, Sherry, von Kaulla and their associates. The symposium also contains valuable contributions on the macromolecular basis of collagen structure by Schmidt and Glimcher.

Taken collectively these two books provide a very useful addition to the modern literature on atherosclerosis. On one hand the former emphasizes the many factors which influence lipid metabolism and will be welcomed by all who work on the metabolic aspects of atherosclerosis. The latter review, with its emphasis on the thrombotic facet of atherosclerosis, will tend to restore the balance which is all too frequently absent in many monographs or colloquia on atherosclerosis.

The books are well written, clearly illustrated and very readable, and will prove useful to all interested in the general aspects of atherosclerosis.

G. S. Boyd

A PHILOSOPHY OF COSMOLOGY

Towards a Unified Cosmology

By Prof. Reginald O. Kapp. Pp. 303. (London: Hutchinson and Co. (Publishers), Ltd., 1960.) 35s. net.

COSMOLOGY is a difficult subject, not only because of the nature of its subject-matter but also because the methods of approach are of a kind with which we are quite unfamiliar in other branches of science, but even among works on cosmology the present book is a difficult one to review.

The main difficulty is the author's insistence on the "Principle of Minimum Assumption". His hypothesis, which is really a hypothesis in the philosophy of science but which he considers to be also a truly scientific hypothesis because he can confirm it in a number of cases, is that in physics the minimum assumption always constitutes the true generalization. As an example, by the minimum assumption for the number of planets in the solar system is meant the unspecific assumption that any number of planets can occur. Now, it is quite possible to call this the minimum assumption if the author so wishes, but this procedure of making the minimum assumption is diametrically opposed to the method actually employed in science, and to the method which many philosophers of science think should be employed, for the minimum assumption is the very hardest one to refute. But many of us believe that the best assumption is the one which lays itself open most readily to possible refutation. However, by means of this hypothesis of the minimum assumption, the author was led to the conclusion that a satisfactory cosmological theory requires a continual creation and annihilation of matter, and he was led to this before the Cambridge cosmologists postulated the continual creation which leads to the steady-state theory. It is puzzling that his assumption should lead to a conclusion which is not so very different from the one to which the Cambridge cosmologists were led by exactly

the opposite view, that is, by looking for the most refutable assumption.

The author does not deal very fully with the difficulty that annihilation of matter may well lead to experimental effects which have not been observed; for example, to the half-life of the proton which one would expect to be able to measure. But I do not wish to be altogether critical of the book. There are many interesting flashes of insight scattered about it, notably a discussion about gravitation. The author lists a set of problems about gravitation; for example, Why should there exist a field which tends to increase its sources, unlike every other field, which tends to decrease them (because like masses attract)? Why, indeed, do like masses attract, and why have we only ever found positive gravitational masses? Why does the inertial mass always occur in association with an active gravitational mass (if, as I would accept, the general theory of relativity merely incorporates the equivalence of inertial and passive gravitational masses)? Why is there only a limited number of constant ratios between unit charge and the inertial mass of the elementary particles? Can one reconcile what is known about gravitation with action at a distance? All these seem to me questions which are worth asking at this particular time.

C. W. KILMISTER

ESSAYS IN COMPARATIVE EDUCATION

The Child, the Parent and the State

By James Bryant Conant. Pp. vi+211. (Cambridge, Mass.: Harvard University Press; London: Oxford University Press, 1959.) 28*s*. net.

SPUTNIK I had a traumatic effect on public opinion in the United States. A scapegoat seemed necessary, and the public education system was selected. Education and educationists—already under fire from several quarters—were increasingly blamed for the apparent failure of the United States to meet the Soviet challenge. Some of the criticism has been virulent, polemical and not infrequently uninformed. Fortunately Dr. James Bryant Conant, the very distinguished scientist, educational administrator, statesman and diplomat, has entered the fray.

Conant's tremendous contribution to the debate in the collection of essays under review is that his criticism of American education is, as befits a scientist, cautious, balanced and thoroughly informed. He chides its more violent critics, but nevertheless examines the problems of American education within the framework of the Soviet challenge to his country. He is not inclined to see it solely, or even principally, in terms of scientific and technological man-power although this aspect cannot be denied. Rather does he accept Comte's view that the great task of education is to rear "a generation of citizens who will be able to rise to the moral and intellectual challenge of these fateful times". In these terms, Conant appreciates that too few American educators have recognized the international post-war role of their country and that too many still continue to believe that "they can live and prosper in an isolated, insulated United States". He sees the larger problems as those of preparing "men and women who can exercise wise leadership" and of developing "intelligent discrimination in future voters". These are formidable tasks indeed, but tasks which Conant admits have been conscious objectives in American education for a long time.

In the face of these problems the process of policy formulation in the United States is one of great complexity. Fundamentally, as the author's title suggests, there is conflict between the interests and demands of children and parents and those of the State. Hence much of Conant's analysis deals comparatively with the mechanics of policy-making in the United States and in Europe. The crucial issue concerns the part the State should play in the maintenance of academic standards, the organization of the curriculum, the training and qualification of teachers, and the finance of schools. Some of Conant's suggestions sound somewhat platitudinous, but one at least is bold. If taken seriously his plea for Federal aid on a large scale for education is bound to provoke controversy. His own proposals regarding the consolidation of 'high schools', the teaching of foreign languages and the place of mathematics and science in the curriculum are moderate and likely to appeal to the practical educator. His support of vocational schools and for some kind of day continuation system is more novel. Many serious educators, however, will think of his proposals as little more than temporary expedients.

Unlike many critics Conant is aware of the difficulties of reforming education. He recognizes, for example, that some policy decisions are not reversible and makes clear that changing conditions in the United States had more to do with the changes in the educational system than irresponsible theorists. In the face of several possible policies from which to choose Conant consistently applies the pragmatic test of asking what consequences—social, political, economic and educational—are likely to flow from the adoption of any one of them. Indeed, one might hazard the guess that he regards the task of prediction as central to the serious study of education. Certainly only through improvements in this field can the study of education grow into a science.

Conant's own method of inquiry—stated by him to be empirical—seems basically pragmatic. His insights are revealing, his analysis of problems acute, and some of his solutions bold. Yet some doubts might be raised about the effectiveness of his comparative approach. The text and very full notes contain much information about the schools of Europe. The appendixes provide a valuable digest of Mr. Khrushchev's memorandum on Soviet education of September 21, 1958, and an extract from the December, 1958, "Law of the Supreme Soviet on School Reorganization". Parenthetically, both documents reveal how universal are some of the problems of education in this technological age. But his analysis of policy formulation in mid-nineteenth century England, present-day Western Germany and the Soviet Union seems dangerously to over-simplify very complex and subtle processes. There are, in brief, rather more sophisticated models on which to base comparative education studies.

Finally, in distrusting discussions about the meaning—and presumably the aims—of education and in eschewing theory in favour of an empirical approach, Conant seems to have failed to distinguish between normative theories and predictive theories. Students of education, in fact, are in real need of the latter and it might be that comparative studies, more than any other type, might make a significant contribution to this task.

BRIAN HOLMES

The Making of the Broads

A Reconstruction of their Origin in the Light of New Evidence. By Dr. J. M. Lambert, J. N. Jennings, C. T. Smith, Charles Green and J. N. Hutchinson. (R.G.S. Research Series, No. 3.) Pp. vii+153+7 plates. (London: Royal Geographical Society, and John Murray (Publishers), Ltd., 1960.) 25s.

THOUSANDS of people know the "Broads". What pleasant memories they evoke—lovely country, interesting bird life, good sailing, joyous parties aboard a motor launch! But how many holiday makers have ever asked themselves how these large lakes were formed? Clearly they are different from any other sheets of water in Britain; How did they come into being? Much research has been devoted to this problem of recent years, and it has been attacked from various points of view—geological, historical, philological, botanical, etc. As the outcome of these investigations it would seem that everything now points to the conclusion that the broads resulted from peat diggings during the Middle Ages. Somewhere about the fourteenth century slight land sinkage, perhaps combined with a tidal break-through such as occurred a few years ago, flooded the peat workings, and this flood water could not drain away owing to the new lower level of the land relative to the sea. The broads, then, were turbaries. It can be shown that, as should be the case, they have vertical peat sides, nowadays somewhat masked by subsequent slight infilling of silt and mud, and we can sometimes also notice transverse baulks of undug peat forming ridges, to-day only just covered by the water; there are also islands of peat similarly only just below water-level. Historical records show that vast peat workings did exist in this area in the early Middle Ages, and the method of working can be determined. Fairly suddenly these methods changed, and much of the peat exploitation ceased. This would seem to correspond with the flooding of the workings.

It must be remembered that transport in the eleventh, twelfth and thirteenth centuries was difficult, and little suitable fuel other than peat existed in East Anglia, which nevertheless was a well-populated part of the country. That peat digging should have existed on a large scale is not surprising. The fact of land sinkage perhaps coupled with tidal breakthroughs, altered the whole picture and resulted in the broads we know to-day. So whether the holiday maker is sailing over aristocratic Ranworth or stately Barton or proletarian Wroxham, let him spare a moment to remember the old peat workers, his forerunners, to whose labours his present enjoyment is due.

MILES BURKITT

Premier Colloque International de Photographie Corpusculaire, Strasbourg, 1er au 6 Juillet, 1957

(Colloques Internationaux du Centre National de la Recherche Scientifique, No. 79.) Pp. 451. (Paris: Centre National de la Recherche Scientifique, 1958.) 5,300 francs.

THE introduction of the photographic emulsion as a detector of charged particles has had an impact on physics which will be familiar to students of evolutionary biology. We can recognize a long and rather obscure development period, a sudden emergence as a practical device, and then an explosive deployment into many fields and applications as its very adaptable features were recognized. In about a decade a vast literature has grown around the subject of nuclear track plates which must bewilder those about to use them for the first time. Nor are there any signs that track plates have reached the final stage of the evolutionary process, namely, the slow decline and ultimate extinction in the face of the competition from newer types of charged particle detectors. This is evident from the subjects covered in this book, which is a record of the First International Conference on Particle Track Photography held at Strasbourg in 1957. The main aims of this conference were to examine the present state of our knowledge concerning the photographic action of charged particles, to survey the results obtained in recent years, and to seek ways of improving the emulsions to meet the changing requirements of physics and the widening fields of application. There are about fifty contributions by delegates from fourteen countries on the theory of latent image formation, the preparation, properties, processing and application of nuclear emulsions. The book is well illustrated by diagrams and photographs and provides a valuable account of the latest techniques written by experts, many of whom were pioneers in the development of track-plate photography.

D. L. ALLAN

The Transits of Venus

A Study of Eighteenth-Century Science. By Prof. Harry Woolf. Pp. xiii+258+11 plates. (Princeton, N.J.: Princeton University Press; London: Oxford University Press, 1959.) 48s. net.

IF one says of this book that it is a careful and scholarly work describing the aims and adventures of those who, in the eighteenth century, tried to determine the distance of the Earth from the Sun by observations of Venus on the rare occasions when the planet was seen projected on the solar disk, one has said almost everything possible by way of general appreciation.

As a contribution to the history of astronomy this is an important work which will be useful primarily as a complete record of the hopes and fears, successes and failures, hair-breadth escapes and solid achievement of the many scientists and travellers who took part in the observational schemes. One can, however, see more in the volume than a catalogue of historical sources. The tale, considered as a whole, shows how essentially modern the eighteenth century was, and how particularly France, Britain and Sweden could even at that time plan great scientific projects in a manner which would not be out of place to-day. The science of the seventeenth century still had recognizable roots in medievalism: that of the eighteenth looked forward with a philosophy still unchanged in our own time. It is even conceivable that we could draw a modern lesson from this record, and by the use of multiple photography of Mercury or Venus when projected on the solar disk overcome the uncertainties introduced by the indefiniteness of the solar limb in any one exposure, and reintroduce the transit method as a modern means for the determination of the solar parallax.

DAVID S. EVANS

Évacuation et Récupération de la Chaleur des Réacteurs Nucléaires

Par Richard Alami et Paul Ageron. (Introduction au Génie Nucléaire, Tome 3.) Pp. ix+246. (Paris: Dunod, 1958.) 2,900 francs.

THIS, the third volume of an introduction to nuclear energy, is devoted to the removal and utilization of heat from nuclear reactors. The work

is directed towards engineering students and those already engaged in the nuclear field who are not specializing in heat transfer.

The first of the four chapters commences with a discussion of the way in which heat is produced in a nuclear reactor. The fundamental laws of heat transfer are next reviewed, and then, after defining the required characteristics of a nuclear coolant, separate sections are given to aqueous, gaseous, liquid-metal and organic coolants. The chapter concludes with a section on aqueous and liquid-metal fuels.

The second chapter deals with the conversion of heat to mechanical energy and includes separate sections on steam and gas turbine cycles. The third covers application of theory to practical reactor systems, some thirteen projects being described according to their coolant classification. The final chapter details methods of determining thermal stresses in simple geometries.

The presentation is attractive, and the text is supported by adequate diagrams and worked examples. Comprehensive tables of physical properties are arranged conveniently within the text. Each chapter includes a reasonable bibliography of supporting references.

The work affords a useful introduction to the subject, but, without implying any criticism of it, it would seem that English readers are already adequately catered for by a number of existing British and American books. R. J. SYMES

Nigeria's Feeding-Stuffs

Their Composition and Nutritive Value. By V. A. Oyenuga. Second edition. Pp. 65. (Ibadan, Nigeria: University Press, 1959.) 8*s*. 6*d*.

AGRICULTURAL progress in all under-developed countries has been delayed because of a lack of knowledge, particularly of the basic facts about the soil and the crops of these regions. Overworked staff in Government research stations have usually been overwhelmed by the need to give all their attention to problems of immediate practical importance. Many of us hoped that the new university colleges would do something to encourage fundamental research, particularly in the sciences relating to agriculture. This book shows one instance of this hope being fulfilled. It is particularly encouraging to find that Nigerian members of staff, like Dr. Oyenuga, are making such a useful contribution to the development of scientific agriculture in their own country.

This book, which is a revision and expansion of the first edition published in 1955, lists the majority of the important foods, both of man and his livestock, which grow in Nigeria. It gives analyses of their composition, including such data as their protein and starch equivalent, and their nutritive value. Useful notes on the cultivation and use of each crop are included. Readers who do not know Nigeria may sometimes be surprised, for example, by such statements as that the water melon has the highest calorific value (658 calories per 100 gm.) of any that has been determined; the mystery is cleared up by the explanation that in Nigeria water melons are grown for their seeds and not for their watery flesh. All who work on problems of nutrition on man or animals in tropical countries, and particularly in West Africa, will find Dr. Oyenuga's book a very useful tool. KENNETH MELLANBY

Australian Native Plants for Home Gardens

By A. E. Brooks. Pp. xvi+149+40 plates. (Melbourne and Sydney: Lothian Publishing Company, Pty., Ltd., 1959.) 45*s*.

THE Australian bush is particularly rich in beautiful floriferous plants fit to grace any garden. Many have already been brought into cultivation, and the aim of this book is to make known to the increasing number of native plant enthusiasts which species are obtainable from nurserymen in Australia and the treatment they require in the garden. The information is presented in dictionary form with brief descriptive and cultural notes arranged alphabetically under the botanical names. Popular names are given also, and an index is provided for them. Articles on such topics as propagation, manuring and transplanting are interspersed, and, together with lists of species flowering at different seasons, or suited to particular conditions, add much to the usefulness of the book. Very few of the plants mentioned can be cultivated out of doors in the British Isles. For the London area they would include one *Grevillea* and one or two ti-trees (*Leptospermum* spp.) among the shrubs, and the golden everlasting (*Helichrysum bracteatum*) and Swan River daisy (*Brachycome iberidifolia*) among the herbs. The paucity of hardy species combined with the inadequacy of the descriptive matter for identification and absence of keys limits the sphere of usefulness of the book to Australia. These deficiencies are to some extent ameliorated by the inclusion of seventy photographs of a high standard by which the majority of the genera are illustrated. R. MELVILLE

Antibiotic Therapy and Staphylococcal Diseases

Edited by Dr. Henry Welch and Prof. Maxwell Finland. (Antibiotics Monographs, No. 12.) Pp. xii+208. (New York: Medical Encyclopedia, Inc.; London: Interscience Publishers, Ltd., 1959.) 4.50 dollars.

THIS volume is No. 12 in the series of "Antibiotics Monographs" and covers the important subject of antibiotics effective against staphylococcal infections.

After a foreword in characteristic style by Marti-Ibanez, the first chapter by Welch deals with the staphylococcal problem and the effects of early antibiotics, penicillin, chloramphenicol, the tetracyclines and bacitracin. The next six chapters deal with erythromycin, oleandomycin, novobiocin, vancomycin, ristocetin, and kanamycin. Each is written by a well-known authority in the use of these antibiotics. The last chapter consists of recapitulation and discussion by Finland.

The book is excellently produced. It gives much useful information about each of the antibiotics considered, full references to their use in clinical practice, an assessment of their effectiveness and discussion of the ease with which drug-resistant organisms arise.

It ends with the conclusion that antibiotics against staphylococci should be used sensibly and only for clear indications, that they should never be used for prophylaxis on a wide scale in hospitals or in closed communities particularly for long periods, that the antibiotics most suitable for each case should be selected and changes made after adequate trial has shown them to be ineffective or inadequate, or when other infections are demonstrated or evidence of toxicity precludes their further use. R. KNOX

ARE THE LAWS OF NATURE SYMMETRICAL?

By Prof. O. R. FRISCH, O.B.E., F.R.S.*

BY symmetry I shall mean mirror symmetry, exhibited by such flat figures as a heart or a fleur-de-lis or the letter A. There are two tests for symmetry. One is to see if a plane can be found—the plane of symmetry—which bisects the figure so that, of the two halves, one is the mirror image of the other. Hence, any figure together with its mirror image in an arbitrary plane constitutes a symmetrical figure. The other test is to form the mirror image of the given figure and see if the two look alike. Children might find it difficult to perform that test since they notoriously confuse *d* and *b*, or *p* and *q* (but would they call them alike if they saw them side by side?). But apart from that the two tests are equivalent, for flat figures; though not for spatial shapes, as we shall see.

Considered as a shape in space, any flat figure is symmetrical, its own plane being the plane of symmetry. The flat shape of a glove is symmetrical, but not the glove itself, the two sides of which are different. Many crystals are symmetrical, but some are not. Many of them will pass the first test—of having a plane of symmetry—and it is easy to prove that they then also pass the second test, of being 'congruent' with their mirror image. But the converse is not true: there are shapes that are congruent with their mirror images but that have no plane of symmetry. This is, of course, well known to crystallographers, but seems surprising when first encountered. Actually we know much more complex forms of symmetry, with several planes of symmetry; but here I am concerned only with the basic difference between symmetrical and asymmetrical shapes.

The important point is that the internal measurements of any shape are not changed when it is mirrored. Hence, for any asymmetrical shape there exist two forms, say, a right-handed and a left-handed one, and the internal measurements are the same for the two. For example, two helices may be drawn on cylinders of the same size, and at the same pitch, and yet be quite different. One is related to our right hand as the other is to our left hand, and so a convention for naming them has come about; but this can be used only by beings who know what we mean by right and left.

So geometry gives no preference to any shape over its mirror image. Does physics? To put it more precisely: any set of geometrical measurements, carried out on a given shape, will give the same results if carried out on its image. Is the same still true if we extend it from geometrical measurements—of lengths and angles—to physical measurements, say, of speeds or forces or temperatures? Would any experimental arrangement give the same result as its mirror image?

The fundamental laws of Newton on which mechanics is based certainly make no distinction between right and left. A weight hung on a string can be made to swing in a circle, and the time for a turn is the same whether it swings clockwise or anti-clockwise. Actually, there is a slight difference because the Earth is turning; this can be eliminated by doing the experiment on the equator, or by just correcting for it. Even then there may be a difference because the string is twisted; so we should strictly use two weights hung by strings which are twisted the opposite way! Anyhow, if all such trivial errors are avoided, the direction of swing makes no difference.

The passage of polarized light through a sugar solution seems to be different. In the absence of the solution, minimum light is obtained with the two Nicol prisms crossed; but with the solution in the light-path, the second prism must be turned to the left to restore darkness. Here we seem to have an asymmetric phenomenon: with the mirror image of this arrangement, that is, with the prism turned to the right, we get brighter light rather than darkness. But the sugar molecules are the cause of that asymmetry; they are twisted, and if we replace them by others which are their mirror image and then turn the prism to the right, we shall once again get darkness, and symmetry is restored.

In the same way we can explain the apparent asymmetry of Oersted's famous experiment, which greatly worried him and his contemporaries. A compass needle above a horizontal wire and parallel to it is deflected when we pass a current through the wire. As before, an apparently symmetrical arrangement gives an unsymmetrical result. But the arrangement is not really symmetrical. The magnetism of the compass needle should be attributed, according to Ampère, to electric currents that go around it. Hence the arrangement is no longer symmetrical; in order to convert it to its mirror image the needle must be turned round, and then the current, of course, deflects it in the opposite sense, as demanded by symmetry.

I hope these examples will make it plausible that the laws of classical physics are indeed symmetrical. Here I mean by classical those phenomena where macroscopic bodies are concerned, made from very large numbers of atoms. But in this century we have learned to observe single atoms, and here a new difficulty appears. An atom passing through a layer

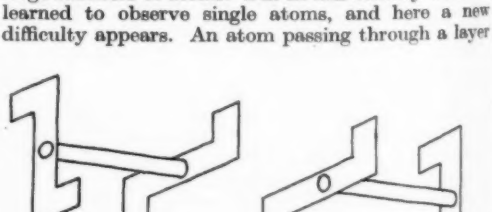


Fig. 1. These two shapes are identical (congruent), as can be seen by turning one by 90° about its rod, and are also mirror images of each other; yet each has no mirror plane

* Substance of an Evening Discourse at the Royal Institution, delivered on May 11.

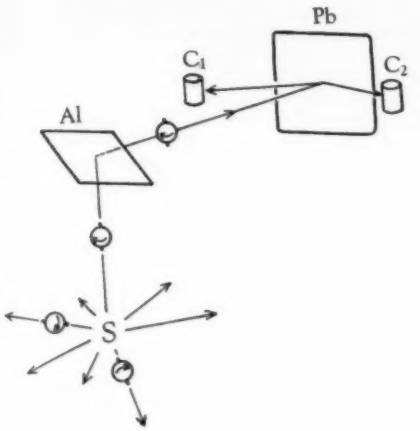


Fig. 2. Electrons from a beta-active source S tend to spin to the left about their direction of flight; that can be shown by scattering them twice, first from an aluminium foil Al and then from a lead foil Pb. The two symmetrically placed counters, C_1 and C_2, then count at slightly different rates

of gas or an electron passing through a metal foil will suffer a random deflexion, either to the right or to the left. Whichever it does, it behaves unsymmetrically in a symmetrical situation. But what else can it do? Its only alternative would be to remain undeflected, like Buridan's ass, which starved between two equal bundles of hay, obedient to the principle of symmetry. A real ass would eat one of the hay bundles at random, and nobody nowadays would worry about its unsymmetrical action. If out of 10,000 asses in that situation 6,000 turned to the right and only 4,000 to the left, then we might deduce that asses behave unsymmetrically; from the action of one ass nothing follows.

So in studying single atoms we must study them many times, or many of them, and analyse the results statistically. Until 1957 (with one single exception) no atomic process had been found to behave unsymmetrically, when averaged over many repetitions. But in 1956 reasons were given to suspect the radioactive beta-decay of unsymmetrical features, and early in 1957 a number of experiments were performed that confirmed this suspicion.

Radioactive phosphorus emits electrons in all directions. If a stream of those electrons that travel vertically upward is made to strike a sheet of aluminium, and those that are thereby deflected by 90° are then made to strike a sheet of lead plate, we should expect that of the electrons that are deflected by the lead plate, equal numbers should go to the right and to the left. But experiment shows that this is not so: about 10 per cent more electrons are recorded on the right than on the left.

Since the arrangement is quite symmetrical, the two positions of the counter represent two experimental arrangements, one the mirror image of the other; yet they give different counting rates. But before I discuss the wider implications of this, I want to describe the mechanism that accounts for this result. It is easy to verify that the electrons reach the lead plate as a symmetrical stream; Why, then, are more of them deflected to the right than to the left? Reversing the lead plate makes no difference, so it must be some property of the electrons. Now, a similar behaviour is seen with a tennis ball that has been given a spin about an axis across its direction of flight: striking a surface normally, it yet tends to go off sideways. The electrons do not, of course, bound back from the lead plate like balls from the floor, but rather like balls from an irregular array of nail heads which represent the atomic nuclei in the lead plate. Even so, the spin does cause an asymmetry in the deflexion pattern, and that asymmetry was indeed calculated by Mott as early as 1929, as soon as Dirac had formulated his mathematical theory of the electron spin.

So the asymmetry we observe indicates that the electrons strike the lead plate with a tendency to spin to the right rather than to the left, like couples performing a Vienna valse. Of course, they were originally travelling upward from the radioactive source before they were deflected by the aluminium sheet. Such a deflexion has little effect on the spin; so we finally conclude that the asymmetry comes from the fact that the radioactive nucleus tends to give each electron a spin to the left on sending it off.

That conclusion could have been reached in 1930: for in that year C. T. Chase found the same asymmetry (see *Phys. Rev.*, 36, 1060; 1930). But the time was not ripe; the effect was small and seemed inexplicable; nobody took notice, and the experiment was soon forgotten. Of course, electrons always spin, but usually in a random manner; and, indeed, when Chase's experiment was repeated with electrons from a hot wire, the asymmetry disappeared (which was erroneously taken to mean that his original experiment had been in error). How then does the nucleus cause the emitted electron to fly off with a definite sense of spin?

To that kind of question physics has no answer, no more than to the question: How does a positive charge attract a negative one? The attraction between opposite charges and many other more complicated electric and magnetic phenomena are fully described by Maxwell's equations; but not 'explained', since that would require their reduction to something more fundamental, and no such reduction has yet been achieved (although Einstein and others have tried). In the same way, the emission of electrons from radioactive nuclei is described in every detail, including the direction of spin, by a theory of which the foundations were laid by Fermi, more than twenty-five years ago.

In its original form, Fermi's theory did not predict any preferential direction of the electron spins; in those days, all physicists believed firmly in the symmetry of the laws of Nature. But it was soon clear that the emission of electrons—or beta-rays, as they were called—from nuclei was in a class apart from other atomic or nuclear phenomena. In quantum theory we compute the probability of transition between an initial state and a final state by assuming a certain interaction between the two states; for those beta-transitions, that interaction turned out to be weaker by a factor of many millions than in other phenomena.

In the past decade or so, several other processes which go by weak interactions have been discovered: the spontaneous transformation of a pion (pi-meson) into a muon (mu-meson), or a neutral lambda-hyperon into a pion and a proton. All those transformations happen within less than a millionth of a second, but that is a long time on a nuclear scale, and the interaction between the initial and the final state is found to be in all these cases just as weak as in beta-transformations.

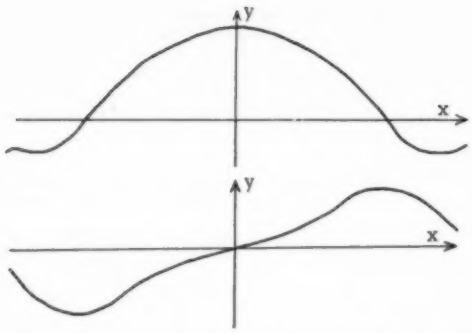


Fig. 3. Graphs representing functions of even parity (above) and odd parity (below)

Of particular interest here were the K-mesons, particles about half as heavy as protons, which were found to break up sometimes into two pions, sometimes into three. This behaviour was in conflict with a well-established rule of quantum mechanics: the rule of 'conservation of parity'. Originally, the parity of a number meant its property of being either odd or even. Later, that concept was extended to apply, not just to numbers, but to mathematical functions. When a quantity y varies in dependence on a quantity x, we call y a function of x, and we can represent a function by a graph. Now if a change in the *sign* of x has no effect on y, we say the function has even parity (or briefly, is even); when it causes y to change sign as well, we say the function has odd parity. The concept is easily extended to the case when f depends on several quantities, say, x, y and z, which may represent co-ordinates in space.

Such functions of space are very important in quantum theory, where they are usually called wave functions, because they resemble waves in special cases. A wave function may have odd or even parity, and it is an important fact that the parity remains unchanged even if the system to which the wave function belongs breaks up (as long as it is not disturbed from outside). This 'conservation of parity' will hold as long as the laws which govern the system are symmetrical, that is, make no difference between right and left, and this was never doubted until about 1956. About that time it became clear that the K-mesons presented a puzzle; it was shown that the system of three pions into which a K-meson can blow up has a wave function with the opposite parity from that of two pions which can also result from the break-up of a K-meson. For a time it was thought that perhaps there were two different kinds of K-meson, having opposite parity; but as the measurements became more accurate and still no other difference between the supposed two kinds could be detected, that idea became unpopular.

In October 1956, T. D. Lee and C. N. Yang, two young, Chinese-born theoreticians working in the United States, published a paper which made history and for which, two years later, they received the Nobel Prize. They looked at the evidence for parity conservation in a variety of processes and found that there was no such evidence for those processes which depended on weak interactions. They proposed some tests, and within a few months they were performed, with positive results. The first tests were quite complex and needed resources found only in a few laboratories; but soon much simpler experiments were devised which proved the simple rule that whenever a particle that has spin is sent out as a result of weak interaction, it tends to spin in a left-handed way about its direction of flight. The particle may be visualized as being like a little left-handed propeller, or that it is fired from a barrel with left-handed rifling, but of course those are just pictures.

Does all this mean that, in her weak interactions, Nature is left-handed, and that her laws are not symmetrical? In a certain sense, yes. But we may widen the concept of symmetry. It has long been known that there is a measure of symmetry between electric charges and the opposite kind. We call them positive and negative, but those are purely conventional labels (and rather unfortunately chosen, since usually it is the 'negative' electrons the movement of which constitutes an electric current). For a time it looked as if the positive charge was definitely associated with the heavy part of matter, the atomic nuclei, and more precisely with the protons which form part of atomic nuclei, and the negative charge with the much lighter electrons. But in 1932 positrons were discovered, which are the positive counterparts of the electrons, and negative protons were first made and observed in 1954. Ever since then, it has been possible to imagine a magic mirror—let us call it a charge mirror—which would leave the shape of an object unchanged but turn all its positive charges into negative ones and *vice versa*. It would even affect some neutral particles: the neutron, which we can image as a small spinning magnet, would be changed into an 'anti-neutron' still spinning in the same direction, but its magnetic poles reversed. In brief, our magic mirror would turn every particle into its anti-particle; but the forces between them would not be changed thereby and the entire system would behave just as before.

But again—just as with a real mirror—this is true only as long as we exclude weak interactions. I said before that any particle emitted as a result of a weak interaction tended to spin to the left about its direction of flight. But if it is an anti-particle that is emitted, then it will tend to spin to the right. That can be verified experimentally: there are radioactive substances that emit positrons rather than electrons, and those positrons are indeed found to spin in the opposite direction.

So the outcome of a weak-interaction experiment is different, according as we change it to its mirror image in an ordinary mirror—which changes right to left—or in our fictitious charge mirror which changes plus to minus. But if we use both mirrors at once then the outcome remains unchanged. To put it in a jocular way: if a physicist had stepped through the looking glass, like Alice, he would be able to tell by measuring the spin direction of electrons emitted in beta-decay, following Lee and Yang; but if at the same time he had stepped into an 'anti-world' where all the particles—including those of his own body—had been transformed into anti-particles, then he could never tell the difference, even with Drs. Anti-Lee and Anti-Yang to help him.

Does such an anti-world actually exist? We do not know yet. When a particle and its anti-particle meet, they annihilate each other and their energy is converted into penetrating radiation. Such radiation is very easily detected, and very little is present on the Earth; hence anti-particles must be rare, not

only on the Earth, but all through our galaxy. But we cannot exclude that other galaxies may be made from anti-matter. Our hope of finding out is based on a careful study of those cases where two galaxies collide (a slow process of interpenetration which may last a million years). If one of them were made from matter and the other from anti-matter, an enormous intensity of gamma-radiation would result which we might observe, directly or indirectly; but so far there has been no conclusive evidence for such radiation. So we do not know yet whether Nature as a whole is charge-symmetrical, with equal numbers of galaxies and anti-galaxies, or whether anti-particles are merely fleeting visitors to our world, created by the clash of high-energy particles and annihilated again almost at once.

To sum up: before 1957 the laws of Nature were symmetrical in two separate ways which we may call space symmetry and charge symmetry. Since 1957 each of these symmetries by itself has ceased to be accurate; but they have become wedded into a vaster, more majestic, symmetry, a truly royal wedding the sight of which delights the philosophical mind.

BLUE HAZES IN THE ATMOSPHERE

By Dr. F. W. WENT
Director, Missouri Botanical Garden, St. Louis, 10

THERE are dozens of everyday experiences which can be cited to substantiate the reality and ubiquity of the blue heat or summer haze, which covers, particularly in summer on sunny and windless days, the whole countryside. We must exclude from this enumeration the haze which is produced in cities by 'smog' (partial oxidation products of petrol vapours) or by smoke from fires or industrial activities. But when we are in the countryside far away from cities, a blue haze is still present, usually much bluer than industrial smokes and hazes. Distant mountains are often blue, and may occasionally become almost invisible in a light blue haze. This is perhaps best illustrated with a description of the Tuscan countryside of the sixteenth century, as given by Leonardo da Vinci in his notebooks.

When reading this, one becomes aware of the number of 'Blue Mountains' (for example, in Australia, where blue hazes over the eucalypt forests are very common) and 'Blue Ridges' (in Tennessee, near the 'Smoky Mountains'), all named after one of the most typical aspects of these mountains: that they are usually enveloped in a blue haze or smoke. This haze occurs more commonly in summer, in areas only little populated, at times when no fires occur.

On warm summer afternoons, the Sun's rays breaking between the clouds can be seen as shafts of light against the dark undersides of these clouds. These rays become visible only because they pass through the even mass of haze. When the blue rays have been filtered out of the sunlight, this light becomes more yellowish (in the tropics the Sun is sometimes called the 'copper cad'), or even red (at sunset). These residual red rays produce the *Alpenglühen*, and the deficiency of blue light at sunset makes all our colour pictures taken at that time too yellow.

There is no better way to observe the blue haze than by flying over the countryside. When flying at a few thousand feet altitude, the fields and forests become indistinct near the horizon. The horizon becomes suddenly clear and sharp when the aeroplane rises above the temperature-inversion layer which, during summer, usually lies at 10,000 ft. The horizon is then formed by the clouds which float in this inversion layer with all haze contained underneath, and depending on the density of the haze, the ground surface is remarkably indistinct. Photographs taken from the aeroplane on days with dense haze are lacking in contrast, whereas on hazeless days, similar pictures are brilliant.

There have been a number of suggestions as to the nature of the haze: (1) smoke; (2) dust; (3) water vapour; (4) fog. Even a summary consideration will show that it can be none of these four possibilities. Concerning (1), these hazes are prevalent over moist tropical uninhabited jungles, over cultivated fields in summer, and in general over places without fires. Besides, the smoke plumes of fires or factories can be followed for dozens of miles downwind where they have not merged completely with the surrounding air. Therefore, the even blue haze cannot have its source in fires. Besides, most smoke is yellow or brown, whereas the common summer haze is blue or grey.

Point (2) can also be dismissed easily. The most pronounced haze occurs on quiet days with very low wind velocities, whereas on windy days hazes are less common. Besides, hazes occur already one day after a good rain, and their prevalence over forests and fields speaks equally against dust. Finally, again the blue colour makes dust an impossibility; dust always has the colour of the soil from which it rises.

The possibility that the haze is either (3) water vapour, or (4) fog, can also be ruled out. In the first place, the haze is most pronounced on warm sunny summer days. It never merges gradually into clouds; these remain always distinct. Water does not have any trace of light absorption in the visible part of the spectrum; water vapour as such can therefore never be visible. It can be observed only after it has condensed into droplets, as fog or clouds. But these droplets are at least 5 mμ in diameter and therefore diffract and reflect all colours equally, making them pure white in sunlight. Smaller water droplets, for example, of 1 mμ diameter, are stable only in strongly over-saturated air, and then they would immediately grow to greater size. Only particles or droplets smaller than the wave-length of light will reflect predominantly the blue rays, and such 0·1 mμ or smaller particles give rise to a blue haze.

We come therefore to the conclusion that blue hazes must consist of very minute, submicroscopic particles. This size precludes their formation by fragmentation of larger particles; breaking any

matter up into sub-microscopic particles requires enormous forces and fantastic amounts of energy, which are nowhere in evidence on the quiet summer haze days.

The only remaining possibility is that these hazes are formed by agglomeration or condensation of molecules already present in the air. This is only possible, in that size-range, by chemical forces, because the kinetic energy of gas molecules at ordinary air temperatures precludes their condensation.

Fortunately several cases are known in which application of light or chemical energy leads to the condensation of gas molecules to blue hazes. The first of these cases was described almost a hundred years ago by Prof. John Tyndall, of Dublin.

He showed that when a beam of intense light was passed through a tube filled with air containing organic vapours a delicate blue light was emitted by that part of the air through which the light had passed. The lower the concentration of the organic vapours, the more slowly the so-called blue cloud formed, and the deeper the blue colour. On continued irradiation the colour of the blue cloud became lighter until it was whitish. Tyndall also found that the air remained optically empty, and that the blue light emitted by the cloud was polarized, indicating that it was reflected by submicroscopic particles, which obviously grew rapidly until they reflected all wavelengths, and thus produced a whitish light.

Another case of experimental blue haze formation has been described and demonstrated by Dr. Arie Haagen-Smit, of the California Institute of Technology. When he mixed dilute petrol or olefine vapours with a low concentration of ozone, a blue cloud (with 'smog' smell) formed. This model experiment indicates what is happening in the atmosphere of a city, where considerable amounts of petrol vapours are emitted into the air, and where the energy of sunlight, catalysed by nitrogen oxides, oxidizes the olefines present.

An experiment, similar to that of Haagen-Smit with olefines, shows that volatile organic substances produced by plants behave in the same way as petrol vapours and ozone. When low concentrations of terpenes are injected into an ozone-bearing atmosphere, a blue haze develops. This is clearly shown in the case of dropping a handful of crushed pine needles, or any other aromatic plant material, into a container with ozone. The crushed leaves soon become enveloped in a slight blue haze, which becomes gradually whiter. This behaviour is exactly like that of organic vapours in the strong light of Tyndall's experiment.

Now it can be shown that considerable amounts of volatile organic substances are emitted into the air in sunlight. A pine forest produces quantities of volatile terpenes, such as pinene, which give the forest its piny smell. The fragrance of meadows is also due to volatile organic substances, and so is the aromatic smell of sagebrush and other types of vegetation of dunes and semi-arid regions. The sweet odour of the forest floor covered with autumn leaves is caused by terpenoid compounds, probably derived from decomposing carotenoids.

The amount of these volatile organic compounds produced yearly by all land-plants and their decomposition products is of the order of 10^8 tons. All this disappears again from the air since only a short distance from forest, meadow, or sage, the aromatic odours are no longer noticeable, at least during day. At night, whiffs of strong aromas may drift for much greater distances, but during the day a mechanism exists which removes the odours. This cannot be a simple oxidation to carbon dioxide and water. Even in the plant no enzymes exist which can decompose terpenes after they have been formed.

But through the experiments of Tyndall with light, and those with ozone, we know that partial oxidation can result in condensation of terpenes to macromolecules which refract the blue light. This is the major—and only demonstrable—mechanism by which the molecularly dispersed (and therefore smellable) aromatic compounds in the air can be deodorified. Therefore the same mechanism explains the removal of terpenes from the air, and the formation of a blue haze.

What happens to the particles which cause the blue haze? We can imagine two mechanisms. One is their oxidation to carbon dioxide and water. But in the first instance, they were formed because oxidation did not result in complete oxidation; and the addition products of the ozonides and peroxides produced by light-oxidation of terpenes are scarcely likely to be more oxidizable than the lower-molecular parent substance.

Therefore, the second possible mechanism is that they continue to grow in size and agglomerate into particles which become large enough so that they can be precipitated with rain or snow. They may then change colour from blue to white, grey, brown, or black. Judging by observable phenomena, the last-named seems to be the actual case. Commonly in the afternoon one finds the upper layer of the blue haze layer to be denser and of a grey or black colour. This is only visible when viewing this layer tangentially, that is to say, from an aeroplane flying in the inversion layer. In addition to this one major thin layer of dark colour, there often are several others at about 1,000-ft. intervals; usually there are one to three, but occasionally as many as seven can be counted. The grey, brown or black colour of these layers indicates that the haze particle size is well above 0·5 mμ, very much above that of the blue hazes under the inversion layer. These denser and darker layers of particles must have originated either in the stratosphere, where we do not have sufficient source materials, or in the haze layers below. This means that the sub-microscopic blue haze particles must grow into 1 mμ size particles in the inversion layer, in a relatively short time-period, measured in hours or days. This presents theoretical difficulties, but the facts seem to be clear-cut. There is some direct evidence indicating the transformation of the blue haze into larger particles.

Whenever a layer of convection clouds (strato- or alto-cumulus) accumulates above a blue haze-saturated air layer, certain phenomena can be observed. There is usually a zone of very active cloud appearance and disappearance in the lower end of the inversion layer; and it is exactly in that zone that very thin, dark grey or black wisps of cloud are seen. I call them 'veil clouds' for apparent lack of a specific name. They are clearly defined along the edges of disappearing strato-cumulus cloudlets. Their dark colour is definitely not due to shadows, for they surround a perfectly white cumulus on all sides. Veil clouds are also visible, not associated with any white clouds, but are always very thin, dark in colour, and occur only in the primary and secondary inversion layers. Frequently the lower surface of strato-cumulus consists mainly or partly of veil clouds. Wherever they extend between the strato-cumulus they remain dark in colour, and therefore I believe

that a considerable part of the darkness of the lower side of strato-cumulus clouds consists of veil clouds, and that their dark colour indicates that their chemical composition differs very much from water. I suggest that veil clouds associated with alto- and strato-cumulus clouds are accumulations and condensations of the blue haze and therefore that they consist of particles of bituminous or asphaltic materials.

At the size the particles reach in veil clouds they may be effective condensation nuclei, and thus may play a part in cloud formation and even in rain production. They might thus partially replace sodium chloride particles as cloud- and rain-forming nuclei.

Only over vegetated areas are blue hazes and veil clouds observed. Over oceans one can see dilute white hazes, apparently consisting of sodium chloride particles, produced by evaporation of droplets of sea water, splattered by rain or splashing droplets into the air. Over absolute deserts no blue hazes occur (usually only yellow dust layers occur there); but over the creosote-covered California semi-deserts, and over the aromatic Australian interior, blue hazes, of perhaps lower concentration than the hazes hanging over Europe or the Mid-West of the United States, are typical. Blue hazes are typical of all tropical vegetated areas, pine forests, and forests in general.

About the fate of the bituminous and asphaltic particles in the veil clouds and grey or black inversion layers, little can be said. It is almost certain that they return to the Earth's surface with rain and snow, become attached to clay and other soil particles, and thus are either washed down with rivers into delta areas, where they might be the source materials for petroleum deposits, or they become incorporated in humus and peat, and provide the basis for much of the heat content of bituminous coals.

I suggest that the blue hazes and veil clouds play a very important part in the heat balance of the Earth and specifically of the atmosphere. They probably absorb about 20 per cent of all the Sun's radiation reaching the Earth. Therefore they should be considered when calculating the heat conversions near the Earth's surface.

There is another interesting aspect of the veil clouds. They must have a considerable potential heat content. Perhaps they provide not only part of the ions needed to build up the enormous potential differences required for lightning, but they might also provide part of the energy for maintaining the multi-mile long lightning shafts. One of the reasons for suggesting this is that the denser the vegetation and the more terpenes volatilize (due to high temperatures), the more intense the thunderstorms are. Over oceans, over deserts, and over the Arctic, they are absent.

Thus it was suggested that volatilization of terpenes and other plant products results in the production of, first, blue haze, then veil clouds, and finally, bituminous materials which, when returned to the Earth by rain and snow, produce the source materials for petroleum formation. The quantities produced are of the right order of magnitude, and all facts seem to fit into a consistent picture, giving a balanced point of view of many, until recently, unrelated facts.

We need proof of many of the statements and suggestions made here before they can be accepted as any more than a hypothesis. But the hypothesis may serve as a basis for further observations on the hitherto neglected blue hazes and veil clouds.

CYTOPLASMIC PARTICLES AND THEIR ROLE IN PROTEIN SYNTHESIS

THE Colloid and Biophysics Committee of the Faraday Society sponsored an informal discussion on this subject which took place under the chairmanship of Prof. J. A. V. Butler, at the University of Reading during March 28–29. The meeting was attended by approximately 150 people. Twenty-three communications were presented; a considerable number of participants contributed in an informal manner to a stimulating and fruitful discussion.

Studies in the field of protein synthesis, which are designed to elucidate some part of the mechanism by which amino-acids are linked to form peptide chains, have become numerous in the past five years. During this period research in this field reached the stage at which a concern with the development of sufficiently simple biochemical systems suitable for examination *in vitro* superseded the employment of whole organisms for investigations on the incorporation of amino-acids into tissue proteins. A similar trend has also occurred in the field of studies on micro-organisms, the cell components of which can also be fractionated and their metabolic behaviour afterwards examined. In view of the large extent to which amino-acids are incorporated into the proteins of the microsomes they are considered to be an important site of protein synthesis in the cell. In micro-organisms the cell membrane and particles inside it are assumed to be functionally analogous to the microsomal structures found in cells of large organisms. Concurrently with the attention given to the problem of protein synthesis in and by microsomes some research workers have been able to make use of other cytoplasmic components, the mitochondria, to demonstrate that isolated mitochondria are capable of forming their own proteins.

The success of metabolic studies on preparations of cell particles and a correct interpretation of such results often depends on some knowledge of the structure of the cell components. Their examination by physico-chemical and microscopic techniques has yielded useful evidence on their structure as well as on the effect on it of different procedures applied for their isolation. It may here be said that one of the purposes of holding this informal discussion at this time was to provide an opportunity for investigators of the dynamic as well as the structural and morphological aspects of protein synthesis to meet and exchange views.

The opening speaker (C. de Duve, Louvain) rightly dwelt at some length on the limits of resolving power of techniques of cell fractionation. This problem is of particular importance in the examination of the

localization of single enzymes, or of enzyme systems. Prof. de Duve discussed the results achieved by his group in applying the technique of centrifugation in a density gradient to the localization of enzymes in tissues. He also reported on another approach, density equilibration in a gradient. This technique makes use of differences in density of particles to separate them in a suitable medium.

Electron microscopy has been of much help in formulating hypotheses which would try to explain some aspects of protein synthesis. It was pointed out by M. S. C. Birbeck and E. H. Mercer (London) that whereas cells such as hepatic cells which synthesize and secrete protein possess an endoplasmic reticulum, that is to say, membranes covered with particles, cells which retain the synthesized protein appear to contain free particles only. Efforts to correlate the site of synthesis with the location of particles or of the endoplasmic reticulum are made difficult by the existence of cell types in which these two points have been observed to be in different parts of the cell.

Many investigators have accepted the particles having a diameter of 15 mμ seen by electron microscopy in the cytoplasm of cells fixed with osmium tetroxide as the structural basis of microsomal nucleo-protein. V. Hanzon (Uppsala) has obtained evidence that these particles may not exist in the living cell since they cannot be detected in pancreatic cells fixed by freeze-drying and subjected to the vapour of osmium tetroxide. These particles seemed to be formed rapidly by a change in the internal milieu of the cell as it is affected by, for example, homogenization or fixation with osmium tetroxide in aqueous medium. Should this observation be confirmed by examination of other cell types it would link up with the results of other investigations which have given evidence that there is no direct relation between the number of visible granules and the content of ribonucleic acid of the microsomes. These ribonucleoprotein particles, now generally referred to as ribosomes, can be examined in the ultracentrifuge as H. E. Huxley and G. Zubay (London) have shown in their studies using *E. coli*. Ribosomes from this organism were found to be able to exist in a number of different stable forms. They could be interconverted by changes in the composition of the medium, particularly in the concentration of free magnesium ions. In addition these workers were able to examine the effect of such changes under the electron microscope. Thus, particles of size 50 *S* have an 'active face' which becomes opposed to another 50 *S* or 30 *S* particle. Examination of 70 *S* particles showed them to be made of two sub-units which resemble 50 *S* and 30 *S* particles, respectively. The pattern shown by ribosomal ribonucleic acid in X-ray diffraction photographs was compared by A. Klug, J. T. Finch and K. C. Holmes (London) to that given by extracted ribonucleic acid. It was considered likely that ribonucleic acid from various ribosomes had its own characteristic configuration; in contrast, the configuration of spherical viruses was probably determined by that of the protein framework.

One effect of chloramphenicol on micro-organisms is to inhibit protein synthesis. S. Dagley and J. Sykes (Leeds) were able to show that in *E. coli* exposure of the cells to chloramphenicol resulted in an accumulation of abnormal species of ribosomes which are apparently useless for protein synthesis. If the antibiotic was displaced from such preparations by magnesium ions, normal ribosomes of size 29 *S* were formed again. The results of an investigation of physical properties of ribosomes from the Jensen sarcoma were reported by M. L. Petermann (New York). The capacity of these ribonucleoprotein preparations to bind protein was found to depend on *p*H or the concentration of magnesium ions, and was observed to be sensitive to changes in ionic strength.

K. McQuillen (Cambridge) was able to report results of an extensive investigation which he and co-workers at the Carnegie Institution of Washington made on time relationships during protein synthesis in *E. coli*. Kinetic studies showed that the smaller ribosomes probably were precursors to the larger. During cell growth the ribonucleic acid and the structural protein of the ribosomes were found to be conserved. Regeneration of ribosomes occurred at an exponential rate in depleted cells; protein, however, was synthesized at a rate proportional to the ribosome content at any time. Evidence was given that radioactivity from amino-acids passed through the ribosomes where it had a transient existence as a protein before it appeared as a soluble protein. The use of ribosomes to study the mechanism of incorporation of amino-acids into proteins has been extended by A. Korner (Cambridge) to preparations obtained from rat liver. These ribosomes prepared by treatment of microsomes with deoxycholate were found capable of incorporating amino-acids on incubation in the presence of adenosine triphosphate and magnesium ions for more than 2 hr.

Most current theories on the mechanism of protein synthesis require the presence of ribonucleic acid from two cytological sources: from the microsomes and from the cell sap (*S*-ribonucleic acid). L. Bosch (Amsterdam) examined the metabolic behaviour of fractions obtained by passing ribonucleic acid through a column of 'Ecteola' resin. Only one of the four fractions obtained by stepwise elution of *S*-ribonucleic acid seemed to be involved in the transfer of amino-acids to microsomes and of radioactivity from phosphorus-32-labelled *S*-ribonucleic acid to microsomal ribonucleic acid. Conversely, only one of three fractions of microsomal ribonucleic acid was found to be capable of accepting radioactivity from phosphorus-32-labelled *S*-ribonucleic acid. The part which *S*-ribonucleic acid might play in protein synthesis was also discussed by V. V. Koningsberger (Utrecht), who reported on the existence in baker's yeast of small, carboxyl-activated peptides originating from activated amino-acids.

F. Gros (Paris) gave an account of experiments in which an alteration in the composition of ribonucleic acid led to modifications in the biosynthesis of proteins. When the uracil analogue 5-fluorouracil was added to growing cultures of *E. coli* some proteins produced were found to have an altered amino-acid composition. The synthesis of new specific protein could also be detected which differed markedly both in biological and physical properties from that produced under normal conditions. These effects appeared to be due to the changes in the ribonucleic acid alone because they also occurred in the presence of thymidine which would make unlikely the incorporation in place of thymine of 5-fluorouracil or its analogues. Another most promising development in the quest to shed light on the role of ribonucleic acid in protein synthesis was discussed by E. M. Martin (London). The use of encephalomyo-

carditis virus, a small ribonucleic acid virus, which can be propagated *in vitro* within Krebs II mouse ascites cells, may make it possible to demonstrate net synthesis of protein. Viral protein can easily be purified and is then available for further chemical study. This system may also allow the study of the effect of previously modified viral ribonucleic acid on protein synthesis. Preliminary experiments designed to examine the time course of events after infection of the host cell indicated that a marked inhibition of the turnover of ribonucleic acid, as measured by incorporation of 6-carbon-14 orotic acid, was followed by the appearance of most of the virus in the mitochondrial fraction.

The incorporation of amino-acids in the protein of whole microsomes requires the presence of the so-called *S*-ribonucleic acid and of certain enzymes from the cell sap. However, evidence has been produced to show that in some microsomal preparations the addition of these factors may be omitted whereas in others different enzymic extracts can replace these '*p*H-5 enzymes' from the cell sap. M. Beljanski (Paris) showed that a soluble enzyme preparation purified from *Alcaligenes faecalis* stimulated the incorporation of amino-acids into the protein of a particulate fraction from the same micro-organism. The same enzyme preparation was capable of replacing *S*-ribonucleic acid and '*p*H-5 enzymes' in a system suitable for the incorporation of amino-acids into microsomal protein from rat liver. This soluble enzyme preparation did not contain those activating enzymes which catalysed the exchange of adenosine triphosphate with pyrophosphate. However, a transfer in the presence of this enzyme preparation of orthophosphate from nucleoside triphosphate to the homologous diphosphate could be observed for the nucleotides of adenine, guanine, cytosine and uracil. If amino-acids were added to this system the formation of peptides was detected. Although the link is not clear between the presence of enzymes which bring about the above-mentioned transfer of orthophosphate to nucleoside diphosphates, and the ability of this enzyme preparation to stimulate the incorporation of amino-acids into proteins, it was of interest to have a report from J.-P. Zalta (Paris) that he could detect this transfer in certain microsomal fractions from rat liver. They were obtained by the action on whole microsomes of detergents of the polyoxyethylene and arylalkylsulphonate type. These fractions were observed to be capable of incorporating amino-acids into their protein without addition of any enzymic system from the cell sap.

The composition of ribonucleoprotein fractions from the microsomes of rat liver has been investigated by P. Cohn and J. A. V. Butler (London). Extraction with acid of these ribonucleoprotein preparations revealed the presence of basic proteins which, like histones, contained about 25 per cent of basic amino-acid residues but possessed a higher content of acidic residues than histones. Some observations on the process of incorporation of amino-acids into various protein fractions of microsomes indicated that, in contrast to results obtained on the living animal, incorporation under conditions *in vitro* apparently occurred to only a small extent in protein fractions which were not associated with ribonucleic acid in the endoplasmic reticulum. Such a connexion between extent and pattern of incorporation into microsomal fractions and the structural integrity of the membranes of the reticulum, and the possible enzymic inhibition resulting from damage to their lipoproteins were suggested by the experiments of A. von der Decken and T. Hultin (Stockholm). The mushroom poison phalloidin was observed to inhibit, under certain conditions, the incorporation into whole microsomes, but not into a ribonucleoprotein preparation which was derived from microsomes by removal of much lipoprotein material with the aid of a detergent. P. Emmelot and E. L. Bennedetti (Amsterdam) were also able to report an inhibition of the ability of whole microsomes to incorporate amino-acids if the rats had previously been injected with dimethylnitrosamine.

Differences in the requirements for the incorporation of amino-acids into proteins of mitochondria and of microsomes were indicated by the paper of D. B. Roodyn (London). The addition of cell sap or of '*p*H-5 enzymes' was found not to be essential, nor was the process of incorporation sensitive to ribonuclease. The distribution of radioactivity in the protein of various fractions obtained by differential centrifugation of mitochondria disrupted by treatment with a non-ionic detergent was reported. M. V. Simpson (New Haven) has employed mitochondria of both rat liver and calf heart to study the incorporation of amino-acids into cytochrome *c*. Mitochondria from calf heart which maintained their ability to incorporate for periods up to 12 hr. permitted the demonstration of a net increase in cytochrome *c* of about 20 per cent during this time. This synthesis required the supply of energy and the presence of amino-acids. It was also observed that amino-acids were incorporated into total protein of a supernatant fluid obtained from mitochondria subjected to ultrasonic treatment.

Much of the available evidence shows that ribonucleic acid is in some manner concerned with protein synthesis. R. W. Hendler (Bethesda) discussed the possibility that complexes between amino-acids and lipids might be involved in the transport of amino-acids and in protein synthesis. Extraction with organic solvents of the oviducts of laying hens, or of the cell wall and ribosomes of *E. coli*, revealed the presence of amino-acids which were bound to lipids in some way. A possible connexion between protein synthesis and the occurrence of amino-acid-lipid complexes was suggested by the pattern of their chromatographic distribution which was altered by processes which inhibit protein synthesis. The existence of such complexes in *Bacillus megaterium* was reported by G. D. Hunter (London), who suggested that their properties would be consistent with those of a precursor of labelled protein. Other studies gave evidence that the primary site of protein synthesis was present in material which sedimented at comparatively low speeds with the cell membrane, after osmotic lysis of protoplasts. A different approach to the problem of the mechanism of protein synthesis in micro-organisms was reported by B. Nisman and H. Fukuhara (Garches, France), who examined the ability of cell fractions of *E. coli* to incorporate amino-acids. Protoplasts were lysed by treatment with digitonin and then subjected to differential centrifugation. Of the two particulate fractions produced, one contained little and the other no enzymes which catalysed the exchange between adenosine triphosphate and pyrophosphate. Both fractions, however, were capable of readily incorporating amino-acids into protein.

P. COHN

NEWS and VIEWS

British Association for the Advancement of Science

The July issue of *Advancement of Science* is noteworthy for a number of reasons. Besides articles on a variety of widely differing subjects by leading authorities, it provides enough information about the forthcoming Cardiff meeting of the Association (August 31–September 7) to whet the appetites of those who will be attending. There is to be a whole-day meeting freed from sectional activities when all members will be able to consider the bewildering world food-population problem. Speakers will include Dr. H. C. Wright, Prof. D. V. Glass, Principal W. A. Lewis, Prof. Dudley Stamp, Sir Alexander Fleck, Prof. H. D. Kay and Prof. P. M. S. Blackett. The needs of young people are to be provided for at Cardiff along similar lines to the admirable junior British Association meetings which have been held during 1960 in Birmingham, Belfast and Sheffield. Besides illustrated lectures and a 'brains trust' there is to be a display of work being done in science classes in many Welsh schools.

Ministry of Aviation : Mr. F. S. Barton

Mr. F. S. Barton retired on August 31, thus terminating some forty years in the public service, almost the whole of it in the radio field. He is the eldest son of the late Prof. E. H. Barton, of University College, Nottingham, who was the only Englishman to work with Hertz in Bonn during 1893, work for which he obtained his doctorate of the University of London. F. S. Barton was educated at Nottingham High School and was a foundation scholar of St. John's College, Cambridge. His Cambridge career was interrupted during the First World War by service with the Royal Naval Air Service Radio Experimental Unit at Cranwell. Returning to Cambridge in 1918, he obtained a first class in the Natural Sciences Tripos and rejoined as a scientific officer the Instrument Design Establishment at Biggin Hill, transferring to the Royal Aircraft Establishment, Farnborough, in 1922. Mr. Barton continued to serve there, his chief interest being in radio control of pilotless aircraft and radio research work, rising to be deputy head of the Department, until he left in 1941 to assume the appointment of director of radio engineering to the British Air Commission in Washington. While there, he served on numerous joint British–American committees and was the vice-president during 1943 of the Institute of Radio Engineers. Mr. Barton returned to Britain at the end of 1946 as director of communications development, becoming afterwards director general of electronics research and development at the time of the integration of the Air and Army sides of electronics research and development in the Ministry of Supply. In the autumn of 1955 he was posted to the U.K. High Commission in Ottawa to inaugurate on a more formal basis the interchange of research and development and production information between Canada and the United Kingdom in the defence field. While in Canada he was during 1954–56 the Council representative for the Institution of Electrical Engineers in the province of Ontario.

Mr. C. J. Francis

Mr. C. J. Francis has been promoted to deputy chief scientific officer in the Ministry of Aviation and appointed to the post of counsellor (Defence Research and Supply) to the United Kingdom High Commission, Canada, in succession to Mr. F. S. Barton. He joined the Civil Service in 1939, and spent the War years in the Directorate of Communications Development, where he had responsibilities for the ground radar chain, radar navigational aids, and blind-bombing aids. During 1945–54 he was at the Telecommunications Research Establishment (now Royal Radar Establishment), and was divisional leader in charge of development of ground search radars for both military and civil applications. In 1947 he became superintendent in the Airborne Radar Department, and was responsible for research and development of blind-bombing systems, Doppler navigational aids, radar reconnaissance systems, and airborne collision warning; for part of the time he was, in addition, head of the Blind Landing Experimental Unit at Martlesham. In 1954 he was appointed to the British Joint Services Mission in Washington, covering the research and development programmes undertaken by the United States Services in the field of electronics. In 1957 he returned to the United Kingdom as assistant director of electronics research and development (air), which post he will relinquish on taking up the appointment in Ottawa.

Mr. M. B. Morgan, C.B.

Mr. Morien Bedford Morgan has been appointed deputy controller of aircraft (research and development) in the Ministry of Aviation. In this appointment—which takes effect in September—he will succeed Dr. W. Cawood, whose appointment as chief scientist to the War Office was announced in July. Mr. Morgan, who has been scientific adviser to the Air Ministry since July 1, 1959, was born in 1912 and educated at Rutlish, Merton, and at St. Catharine's College, Cambridge. At Cambridge he obtained first-class honours in the Mechanical Science Tripos and won the Seely Prize in aeronautics in 1934. After an apprenticeship with Mather and Platt, Ltd., he joined the Royal Aircraft Establishment, Farnborough, in 1935, where as a member of the Aerodynamics Department he was engaged on general flight research and development, specializing on problems of stability and control. He was appointed head of the Aerodynamics Flight Section at Farnborough in 1946, and in 1948 was made head of the newly formed Guided Weapons Department. He held this post until January 1954, when he took over as deputy director (air), Royal Aircraft Establishment, a post he held until 1959. In 1957 he was awarded the Silver Medal of the Royal Aeronautical Society for his contributions in the execution and direction of aeronautical research and development. He has maintained a keen interest in flying—he obtained his pilot's '*A*' licence in 1944.

Animal Husbandry at Bristol : Prof. T. K. Ewer

PROF. TOM KEIGHTLEY EWER, at present professor of animal husbandry in the University of Queensland, has been appointed to the newly established chair of animal husbandry in the Veterinary School of the University of Bristol. Prof. Ewer, who will take up his appointment early in 1961, was born and educated in England and emigrated to Australia. He graduated in the Veterinary School of the University of Sydney in 1937, having previously obtained the H.D.A. with honours at the Hawkesbury Agricultural College. During the war years Prof. Ewer held appointments with the New Zealand Department of Agriculture and at the Canterbury Agricultural College, and was engaged on work on nutritional and parasitological problems of sheep. In 1947 he was awarded a Wellcome research fellowship by the Animal Health Trust which enabled him to conduct work on ovine rickets in the Institute of Animal Pathology at Cambridge, where he was awarded the degree of Ph.D. Prof. Ewer accepted the appointment he now holds in 1950 and was Dean of the Faculty of Veterinary Science in the University of Queensland for five years. In recent years he has carried out two assignments with the Animal Production Branch of the Food and Agriculture Organization—one in Venezuela, the other in Burma. Prof. Ewer's list of publications is in the field of nutrition in the sheep, his current interest being in the use of sorghum silage and various protein and non-protein nitrogen supplements.

National Academy of Sciences–National Research Council : Appointments

THE following appointments as chairmen of divisions of the U.S. National Academy of Sciences–National Research Council have been announced : Prof. Robert C. Elderfield, professor of chemistry in the University of Michigan, as chairman of the Division of Chemistry and Chemical Technology ; Dr. Edward P. Espenshade, jun., chairman of the Department of Geography at Northwestern University, as chairman of the Division of Earth Sciences ; Dr. Emil W. Haury, professor and head of the Department of Anthropology at the University of Arizona, as chairman of the Division of Anthropology and Psychology ; Prof. J. Barkley Rosser, professor of mathematics at Cornell University, as chairman of the Mathematics Division.

The Henry Bryant Bigelow Medal

THE first Henry Bryant Bigelow Medal for oceanography has been awarded to the man it honours. Dr. Bigelow, one of America's foremost marine scientists, was among the founders of the Woods Hole Oceanographic Institution. He was its first director, served later as president of the Corporation and is now chairman of the Board of Trustees. He is also professor emeritus of zoology at Harvard University, having taught there for nearly half a century, and is a research oceanographer and former curator of the Museum of Comparative Zoology at Harvard. The Bigelow Medal was established earlier this year by the Trustees of the Oceanographic Institution without the knowledge of their chairman. It is designed with a likeness of Dr. Bigelow on the face and the Institution's first and most famous research vessel, *Atlantis*, on the back. With it go a citation and a cash prize of 2,500 dollars. It is to be awarded from time to time to those "who make significant inquiries into the phenomena of the sea".

Cloud Pictures from Satellite *Tiros* I

THE March issue of the *Monthly Weather Review*, published by the U.S. Weather Bureau (on June 15), contains a number of fascinating photographs of cloud systems taken from the satellite *Tiros* during April 1960. The height of the satellite varied between 436 and 460 miles. The photographs are of the cloud systems of temperate-zone depressions over the Atlantic, Pacific and Mediterranean and a tropical cyclone north of New Zealand. The synoptic charts are printed alongside, and the correspondence between the cloud belts and the familiar curved fronts of the weather maps is most striking. The photographs show three major systems, the cloud belts within the systems, and cloud areas within the belts. Both single photographs and mosaics covering large areas are reproduced. There can be no doubt that cloud photographs transmitted from artificial Earth satellites will be a major tool of the weather forecaster of the future.

Intergovernmental Conference on Oceanographic Research

SCIENTISTS and government officials from thirty-four countries meeting in Copenhagen during July 11–16 at the invitation of Unesco have agreed to place the whole subject of oceanography on an international footing. Recognizing the importance of oceanographic research for the world as a whole, they recommended that Unesco set up a permanent intergovernment oceanographic commission to organize concerted action by States and by international bodies. Hitherto, the main obstacle to the development of oceanographic research has been the lack of qualified oceanographers. Oceanography has been somewhat of a poor relation among the various branches of science and the number of laboratories and university courses devoted to it is relatively very small. Oceanography now requires large-scale international co-operation. The Conference therefore recommended a number of measures, the chief of which is that an intergovernment oceanographic commission be set up and work with a special Office of Oceanography within the Department of Natural Sciences of Unesco to co-ordinate research on a permanent basis, the Commission meeting, in principle, once a year. This is subject to approval by the 82-nation General Conference of Unesco which meets in Paris next November.

The shortage of oceanographers, considered as one of the main obstacles to oceanographic research, led to the recommendation that the number of fellowships financed by Unesco be appreciably increased so as to offer greater training facilities for such specialists, particularly for those countries in which oceanography is little developed and which wish to participate in oceanic research. Such training should include participation in expeditions organized by the various countries or by Unesco and in short lecture courses or training courses in research institutes and institutions of higher education. Unesco is also asked to organize a conference to discuss the means of developing teaching in the marine sciences in universities. Because the training of oceanographers is so urgent, the Conference recommended that the money proposed in Unesco's budget for 1961–62 for the operation of an international oceanographic vessel be used for other training activities. Other steps towards

international co-operation recommended by the Conference in a series of resolutions are the standardization and intercalibration of methods and equipment—such as the sizes and types of nets and the techniques used in the collection of plankton; the collection and exchange of oceanographical data; and the organization of joint research programmes in specified regions by groups of countries, such as that planned for the Indian Ocean. The Conference also asked Governments and international organizations to take immediate steps to prevent pollution by radioactive material and to intensify their research programmes in this field.

Cosmic Radiation Studies in South America

The Latin American Council of Cosmic Radiation, with members from Argentina, Bolivia, Brazil, Chile, Mexico, Peru, Puerto Rico and Venezuela, met during August 1–3 at the Centro Brasileiro de Pesquisas Físicas in Rio de Janeiro. Observers representing the International Union of Pure and Applied Physics and Unesco were also present. The meeting was called and organized by Unesco's Centre of Scientific Co-operation for Latin America, Montevideo. The Council decided to organize a brief course of three weeks duration and seminars to be held in Mexico beginning late in June 1961. The latter will cover the following topics: composition of primary cosmic radiation, physics of interplanetary plasma, solar physics, time variations, extensive air showers and theory of geomagnetic effects. An effort will be made to secure the participation of physicists from other than Latin American countries in these seminars. The Council hopes to send through proper channels suggestions for experiments which can be made aboard space vehicles and artificial satellites. Members of the Council attending the Rio meeting were Prof. José Manzano (Argentina), Dr. Narayan Nerurkar (Bolivia), Prof. Georges Schwachheim (Brazil), Prof. Gabriel Alvial (Chile), Prof. Manuel S. Vallarta (Mexico) and Prof. Manuel Bemporad (Venezuela). Observers present were Prof. Gabriel Fialho (International Union of Pure and Applied Physics), Dr. Angel Establier (Unesco), Prof. Gleb Wataghin (University of Turin) and Prof. Gianpietro Puppi (University of Bologna). Prof. Vallarta acted as chairman.

Fossil Meteorites

Additional information is provided by B. I. Vronsky (*Priroda*, **9**, 116; 1959) on the probable 'fossil meteorite' (*Nature*, **184**, 1026; 1959). This meteorite was discovered in November 1957 in the valley of a small river, Zarya, a tributary of the Susuman River, itself a tributary, Berelekh, Magadan district of Siberia. This meteorite, which received the name 'Susuman' and which weighed about 19 kgm., was discovered at the base of an alluvial deposit consisting of gravels and mud. Thus, in age it is assumed to be Post-Glacial and about 15–20 thousand years old. It is only now that it has been made known that in this region, a few years previously, two similar meteorites have been discovered in similar situations. Thus, the iron meteorite, 'Mal'dyak', was discovered at a depth of 4·6 m. in gravels of the River Mal'dyak situated 28 km. to the south of Susuman. In 1941 the iron meteorite 'Burgavli', weighing 24·9 kgm., was found in the gravels of the River Adachi, a right tributary of Yana River. These three meteorites are now being studied in detail.

Industrial Health in Britain

Mr. R. Carr asked in the House of Commons for a statement about the development of industrial health services in Britain. The Minister of Labour, Mr. E. Heath, said in reply on July 25 that the development of such services is under consideration by the Industrial Health Advisory Committee. Legal requirements safeguarding the health of industrial workers are contained in the Factories Acts and Regulations, which are constantly being revised and extended. In addition, many firms voluntarily provide medical and nursing services, and to encourage more employers to do so, a booklet is to be published later this year giving details of the cost of some of these services, together with the views of management and work-people about them. Group schemes have also operated successfully in a few places with special circumstances, such as Slough and Harlow, and we now need to explore the possibilities of establishing group schemes in areas of a different kind. Another need is the further use and development of industrial hygienic services to carry out chemical, physical and biological tests where special hazards exist or are suspected. The Minister said that arrangements are being made to set up an organization to collect information about existing facilities regularly and to make it available to employers. He had discussed further action with the Nuffield Foundation, which has had considerable experience in promoting industrial health schemes, and the Trustees of the Foundation are allocating £250,000 for the further development of group industrial health schemes and industrial hygienic schemes. The money will be used to assist practical schemes which promise to become self-supporting, and in selecting such schemes the Foundation would work in the closest co-operation with the Ministry of Health.

Preparing Teachers for Health Education

Health education is not always recognized as a discipline of its own and given its appropriate place in the training of teachers. A report of a joint World Health Organization/Unesco Expert Committee (World Health Organization: Technical Report Series. No. 193. 1960. 1*s*. 9*d*.) provides a flexible framework of guiding principles for such training, which should take place not only through courses of instruction but also through the teacher's actual mode of life in his training institution, his contact with student health services, and his experiences when practising teaching. Health education in schools should be based on the health needs and interests of the children, the teacher having opportunities for imparting it, both directly and by the part he can play in the school health services and in planning a healthy school. To enable him to make the most of his opportunities he must have some knowledge of growth and development, personal and community health, health practice in schools, and methods of health education. The report discusses these subjects, the future teacher's life as a student in relation to his opportunities for health work and to health services for students, practice teaching and the education he acquires as a fully-fledged teacher. Other sections deal with planning of training for teachers in health education, the desirability of one person in each training institution being responsible for co-ordination of courses and activities in health education, and possible future national and international developments, studies and research in the subject.

The Research Defence Society

THE Research Defence Society policy of presenting factual information in readily assimilable forms on 'explosive' topics like immunization, vaccination and animal experiments continues with an excellent pamphlet on vaccination against whooping cough, diphtheria and tetanus (*Conquest* Pamphlet No. 11. Research Defence Society, 11 Chandos Street, Cavendish Square, London, W.1. 6*d*.). The methods of immunization against these three conditions and the problems arising in connexion with the procedure are considered together for two reasons: the first is that protective injections against these diseases are now usually given earlier in life than are most others; and the second that these substances are often given all together as a mixture, thus minimizing the number of injections required. The value of the pamphlet is that in no cases are dangers minimized or awkward questions evaded. These are put simply, answered objectively, and include information which indicates where preventive medicine may not always have been effective. The pamphlet should be widely distributed in maternity and child welfare clinics.

Genetic Recombination in *Salmonella typhimurium* occurring during Transmission of Colicinogeny

AN interesting item in the annual report of the Lister Institute for 1960 is an investigation by Drs. Ozeki and Howarth into the recombination of chromosomal genes in systems in which colicinogeny is being transmitted. Their results indicate that, in about one pairing in a million, genes believed to be located in the bacterial chromosome (for example, genes regulating various biosynthetic steps and a gene for resistance to streptomycin) undergo recombination. Recombinants can be detected in small numbers whenever colicinogeny-1 is being transmitted at high frequency, and in larger numbers when colicinogeny-*E*1 is also being transmitted. Many pairs of genes cannot be simultaneously transduced by a single phage particle, probably because they are not close together in the bacterial chromosome, only a very small fraction of which can be enclosed within a single phage particle. A number of crosses involving such pairs of apparently unlinked genes were made by the newly discovered method of hybridization, and in every instance a fraction of the recombinants was found to have received both pairs of genes concerned from the strain which was transmitting colicinogeny. This suggests that, in this new kind of hybridization, either the whole or at least a large fraction of the chromosomes of the transmitting strain is enabled to recombine with the chromosome of the acceptor strain, in contrast to the situation in phage-mediated transduction. It seems likely that the new method of hybridization may prove of great value in determining the arrangement of the genes along the whole length of the *Salmonella* chromosome, a problem which could not be investigated with the previously available method of hybridization by phage-mediated transduction.

Nature Conservation in East Anglia

A BOOKLET, "Nature and Conservation in East Anglia", produced on the occasion of the Duke of Edinburgh's visit on July 8, 1960, describes briefly the work of the Conservation Corps of the Council for Nature in clearing scrub for the National Trust at Wicken Fen, the Nature Reserve at Havergate Island and Orfordness, and the work of the Field Studies Council at Flatford Mill (pp. 16. London: The Council for Nature, 1960). This attractive booklet also gives brief accounts of the work of the Council for Nature and the Royal Society for the Protection of Birds as well as of the Nature Conservancy, and is well designed to promote public awareness of our heritage of wild life and the need to conserve it more effectively.

Darwin's Notebooks

IN Part 3 of "Darwin's Notebooks on Transmutation of Species", Sir Gavin de Beer shows that the contents of the "Third Notebook" owe nothing to Malthus's work (*Bull. Brit. Mus.* (*Nat. Hist.*), Historical 2, No. 4. London, 1960). "It is therefore of great importance to note that Darwin hit on the principle of natural selection independently. After a consideration of changes resulting in the formation of new species, he wrote: 'All this agrees well with my view of those forms slightly favoured getting the upper hand and forming species'." Darwin himself realized the historical importance of these lines, because at a later date when he looked through his "Notebook", he wrote at the head of it: "Towards close I first thought of selection owing to struggle". How the stimulus of Malthus's book put Darwin on the scent of natural selection is well brought out in this edited version of his "Third Notebook".

Lancaster Royal Grammar School Natural History Society

THE extra-mural duties involved in running a society in a grammar school is an arduous task for the masters and boys concerned. Lancaster Royal Grammar School may be rightly proud of its Natural History Society, which has recently celebrated its twenty-first anniversary. The society periodically publishes a magazine, and, in the ninth issue of *The New Biologian*, the contributions include articles by the masters and boys, records of the Society's activities, etc. The articles include descriptions of biological stations and field study centres and show the value of these in awakening genuine interests among the participating students. One clear difference emerges from the journal and those of natural history societies established in boarding schools: almost invariably in the latter their journals contain some record of original work by boys. Biology masters in grammar schools would do well to examine them.

The Korsakov Journal of Neuropathology and Psychiatry

ARRANGEMENTS have been completed for the quarterly publication of an English translation of selected papers from the well-known Soviet periodical, *The Korsakov Journal of Neuropathology and Psychiatry*. The English edition will begin with the first issue of 1960, and will contain English abstracts of all articles not translated. The translated journal will be published by Pergamon Press, and Dr. W. Ritchie Russell (Oxford) will act as English editor.

American Society for Cell Biology

A GROUP of cell biologists met in New York on January 9 and again on May 28 of this year, and organized a new society, the purpose of which is to further the development of cell biology and to improve communication among scientists representing diverse disciplines. Membership is expected to include biochemists, biophysicists, cytologists, histologists,

microbiologists, physiologists and others having in common an interest in the cell. The organization has been named the American Society for Cell Biology. Applications to join the Society should be sent to Dr. Montrose J. Moses, Box 2982, Duke University School of Medicine, Durham, N.C. Membership is not restricted to residents of the United States.

Maccabæan Prize and Medal

THROUGH the generosity of the Maccabæans, the Faculty of the History of Medicine and Pharmacy of the Worshipful Society of Apothecaries is able to offer an annual prize of not less than 25 guineas, with a bronze medal, for the best original essay on a subject connected with the history of medicine or pharmacy written by an undergraduate or graduate less than thirty years of age. Further information can be obtained from Dr. F. N. L. Poynter, The Wellcome Historical Medical Library, 183 Euston Road, London, N.W.1.

Research in Biochemistry and Allied Sciences

A COLLOQUIUM on the Organization and Financing of Research in Biochemistry and Allied Sciences is to be held under the joint auspices of the Biochemical Society and the British Association, in the Physiology Institute, Newport Road, Cardiff, on September 7 (the last day of the British Association meeting) at 2 p.m. It is expected that the following speakers will take part: Prof. John Pryde (chairman), Prof. A. Haddow (British Empire Cancer Campaign), Dr. F. J. C. Herrald (Medical Research Council), Sir William Slater (Agricultural Research Council), Mr. C. Jollife (Department of Scientific and Industrial Research), Dr. G. F. Marrian (Imperial Cancer Research Fund), Prof. F. C. Happold (University of Leeds), Dr. F. A. Robinson (on behalf of industry), Dr. J. B. Bateman (U.S. Embassy, London), Prof. A. Neuberger (St. Mary's Hospital Medical School). The Colloquium is being organized jointly by Dr. K. S. Dodgson, Physiology Institute, University College, Newport Road, Cardiff, and Dr. W. J. Whelan (meeting secretary of the Biochemical Society), from whom particulars can be obtained.

The Night Sky in September

FULL moon occurs on Sept. 5d. 11h. 19m. U.T., and new moon on Sept. 20d. 23h. 13m. The following conjunctions with the Moon take place: Sept. 1d. 08h., Saturn 4° S.; Sept. 12d. 01h., Aldebaran 0·2° S.; Sept. 13d. 10h., Mars 5° N.; Sept 22d. 22h., Venus 3° S.; Sept. 27d. 10h., Jupiter 5° S.; Sept. 28d. 14h., Saturn 4° S. In addition to these conjunctions with the Moon, Venus is in conjunction with Spica on Sept. 20d. 22h., Venus being 3° N.; and Mercury with Spica on Sept. 26d. 16h., Mercury being 1° N. There will be a total eclipse of the Moon on September 5 and a partial eclipse of the Sun on September 20–21; both eclipses will be invisible at Greenwich. There were two eclipses in March of this year, making in all four in 1960. As is usually the case when four eclipses occur in a year, they take place in pairs about six months apart. Mercury and Venus are too close to the Sun for observation. Mars rises at 22h. 15m., 21h. 50m. and 21h. 20m. at the beginning, middle and end of the month, respectively. It is in Taurus at the beginning of September and moves into Gemini on Sept. 20. Mars is rapidly becoming brighter, its stellar magnitude being +0·5 and its distance from the Earth 106 million miles in the middle of the month. Jupiter sets at 22h. 40m., 21h. 50m. and 20h. 55m. on September 1, 15 and 30, respectively; it is in Ophiuchus, but conditions are not very favourable for observation. Its stellar magnitude is −1·8 and its distance from the Earth on September 15 is 470 million miles. Saturn is in Sagittarius, east of Jupiter, and sets at 0h. 00m., 23h. 05m. and 22h. 05m. at the beginning, middle and end of the month, respectively. It is stationary on September 15 and afterwards resumes eastward motion among the stars. Its stellar magnitude is +0·6 and its distance from the Earth in the middle of the month is 890 million miles. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Sept. 9d. 4h. 45·1m., ξ^1 Cet. (*D*); Sept. 12d. 1h. 14·3m., 89 Tau. (*R*); Sept. 12d. 1h. 21·3m., σ^2 Tau. (*R*); Sept. 13d. 1h. 05·0m., 117 Tau. (*R*). *D* and *R* refer to disappearance and reappearance, respectively. The autumnal equinox occurs on Sept. 23d. 01h.

Announcements

MR. M. D. TAYLOR, of the Department of Chemical Engineering, King's College, University of Durham, has been awarded a CJB Scholarship, of the value of £450 per annum, for two years in the first instance. This scholarship scheme, administered jointly by Constructors John Brown, Ltd., and the Institution of Chemical Engineers, is to encourage research in chemical engineering and, in particular, in chemical plant design.

AN International Symposium on Photoelasticity will be held during October 29–31, 1961, at the Illinois Institute of Technology, Chicago, Illinois. Further information can be obtained from Dr. Paul D. Flynn, Illinois Institute of Technology, Chicago 16, Illinois.

A SYMPOSIUM on Redox Processes will be held at the Chemical Laboratories, University of Allahabad, under the auspices of the Chemical Research Committee, Council of Scientific and Industrial Research, India, during January 1961. Further information can be obtained from Dr. Satya Prakash, Department of Chemistry, University of Allahabad.

A CONFERENCE on Diffraction will be held during November 9–11 at the Mellon Institute, Pittsburgh, Pennsylvania. Sessions will be devoted to metals and alloys, instrumentation, structures, polymers and fibres, refractories, electron probes and electron diffraction. Further information can be obtained from L. F. Vassamillet, Mellon Institute, 4400 Fifth Avenue, Pittsburgh 13, Pennsylvania.

THE Institution of Electronics, with the co-operation of the Hull Electronic Engineering Society, is to hold an Electronics and Instruments Exhibition and Convention at the Farmery Hall, George St., Hull, during the period September 28–30. Admission will be free of charge and by tickets obtainable from exhibitors, or from W. Birtwistle, General Secretary, Institution of Electronics, 78 Shaw Road, Rochdale, Lancs., or from C. W. Wyan, Honorary Secretary, Hull Electronic Engineering Society, 2 Lockton Grove, Calvert Road, Hull, Yorks.

ERRATUM. In the communication entitled "Structure of β-Acylacrylic Acids", by Prof. Nils Hellström, in *Nature* of July 9, p. 146, the description of the ordinate of Fig. 1 is incorrect; for "Acid/sodium hydroxide" read "Sodium hydroxide/acid".

THE FOURTH INTERNATIONAL GOITRE CONFERENCE

THERE is a growing body of opinion in favour of replacing the large international conferences, attended by three or four thousand people, by smaller gatherings of an international character, and devoted to more specialized topics. At such meetings there should be better opportunity for expert discussion of communications, less overlapping of programmes and, above all, a chance of seeking out and talking with specific participants. Confirmation of the correctness of such expectations was afforded by the very successful meeting of the Fourth International Goitre Conference held in London during July 4–8. In all, about 600 endocrinologists, clinicians and biochemists, drawn from more than forty countries, took part. More than a hundred papers were delivered, and a book of abstracts of about 500 words each, in four languages, was provided at the time of registration.

A very large part of the subject-matter reported at the Conference was devoted to endemic goitre, and papers were read which surveyed its incidence in various parts of the world, the genetic aspects of the subject, and the part which goitrogens and other factors might play in its etiology. That iodine deficiency, in the opinion of Perinetti *et al.* and Kicic *et al.*, is the most important cause of endemic goitre was shown by the reliable effects which iodized salt had brought about in the disappearance of the condition in such different areas as Mendoza in Argentina and Gornja Josanica in Yugoslavia. On the other hand, Kilpatrick *et al.* suggested that iodine deficiency probably plays only a part in the development of goitre in an area to the west side of Sheffield, which was compared with a coastal district near Edinburgh. Although the iodine content in the water supply in the two districts is not dissimilar, there was a higher incidence of goitres in Sheffield. More fish was consumed in Scotland. It was further noticed that there was a definite familial linkage in the disease. An examination of the iodine content of goitrous thyroids did not reveal consistently low values as compared with normal thyroids. Observations of the seasonal incidence of goitre in sheep and cattle and the similar seasonal variation in anti-thyroid activity of milk (even in dried milk) suggests that though iodine may play a part in the incidence of goitre in the Sheffield area, goitrogens may also be a contributory factor.

Similar views were put forward by Costa *et al.*, who conclude that endemic goitre is a process which goes beyond mere alteration in thyroid function and that it is not a simple adaptation by the gland to iodine shortage. In a very careful examination by Koenig in Switzerland of nineteen endemic cretins, ranging in ages between 46 and 93 years, a number of interesting facts arose. It is difficult to find cretins born in Switzerland after 1910, which is at least ten years prior to the inception of iodine prophylaxis. Results of determinations of protein-bound iodine and the uptake of thyroidal iodine-131 show wide individual variation. In most cases these values were low, but in some they were normal and in others high. Some cretins responded well to exogenous thyroid-stimulating hormones, while others did not. The basic metabolic rate was a most unreliable indication of the state of affairs.

Cassano *et al.* believe that iodine deficiency may arise from excessive renal loss. They found that a high rate of excretion of iodine normally occurs in puberty and pregnancy and, significantly, in Cushing's disease and acromegaly, suggesting a hormonal basis for the increased renal excretion. The hormone most active on renal clearance of iodine is aldosterone. In any areas where iodine deficiency is likely, increased renal clearance of iodine, as in pregnancy and puberty, might favour the development of goitre.

A number of *Brassicae* are goitrogenic when fed to animals. Astwood *et al.*[1] isolated L-5-vinyl-2-thiooxazolidone (goitrin) from yellow turnip, but this cyclized derivative of an *iso*thiocyanate does not exist as such in the plant but is formed enzymically from progoitrin, identified by Greer as a mustard oil glycoside. It is now reported (Greer *et al.*) that the conversion to goitrin can be accomplished by microorganisms in the animal gastrointestinal tract. Both cooked and raw brassicaceous vegetables are therefore potentially goitrogenic, the effect being an iodine-blocking one.

Endemic goitre is not always associated with a deficiency of iodine in the soil and a relation to pasture has been suspected. Thus, in Tasmania endemic goitre occurs and it has been shown that cows fed on the brassica 'chou-mœllier' secrete an active goitrogen in their milk which is consumed in quantity by school children. Peltola reports a somewhat similar situation from Finland, where endemic goitre areas exist. Recently published researches of Bachelard and Trikojus[2] go far to elucidate this problem. They find the turnip weed *Rapistrum rugosum*, which grows abundantly in certain areas of Australia, to contain a glycoside of cheiroline (γ-methylsulphonylpropyl-*iso*thiocyanate). Cheiroline had a variable goitrogenic effect when administered to rats, suggesting its possible transformation into an effective goitrogen in the animal body. Incubation of cheiroline with cows' ruminal contents afforded *sym*-di-cheiroline thiourea $(CH_3SO_2[CH_2]_3NH)_2C{=}S$. This interesting transformation may occur more generally with *iso*thiocyanates and be of importance in bringing about endemic goitre.

Dimitriadou *et al.* believe that there may be a defective synthesis and storage of fully iodinated hormones in some cases of non-toxic goitre and that this might be due to a defective iodinating mechanism. By means of their 'supplemented iodine-131 test' it was possible to calculate the amount of iodine-127 available to the thyroid, the rate at which iodine-131 became fixed organically into the various iodinated amino-acids in the gland, and also to estimate the size of the thyroid iodine stores. Results indicated little difference in urinary iodine excretion between patients with thyrotoxicosis, those with non-toxic goitre (with or without avid uptake of iodine) and euthyroid individuals. Stores of iodine compounds in the thyroid were diminished in cases of non-toxic goitre and the rate of transfer of iodine-131 into heavily iodinated compounds, that is to say, diiodotyrosine and the iodinated thyronines, was slower in such persons. This defective or slowed iodinating mechanism can be compensated for, however, by iodine supplements even though, by normal standards, these persons were not deficient of iodine in the diet.

A number of authors reported studies in which family pedigrees were examined to discover the incidence of abnormal thyroid behaviour. Fraser *et al.* and Thould and Scowen reported on the condition known as Pendred's syndrome, the main features of which are bilateral deafness, observed early in life, and goitre developing from about eight years onwards. The defect, which is probably congenital lack of a peroxidase enzyme system in the thyroid, results in inadequate organic binding of iodine. The condition is rare, and genetic data suggest that the syndrome is inherited in a recessive manner.

Other genetic studies were reported on simple goitre. One pedigree of 250 individuals was studied (Van Wyk *et al.*) which suggested that the occurrence of goitre in this family was a simple dominant trait which is more commonly overt in the female. Serologically there was a characteristic abnormality which consisted of a protein-bound iodinated constituent migrating electrophoretically with the albumin fraction. Other studies in a different family provided evidence of defective peripheral dehalogenation of iodotyrosines.

Elucidation of the process of biosynthesis of its hormone by the thyroid gland has proved to be a peculiarly baffling problem. The process may be divided into the three stages of concentration of iodide from the blood (the 'trapping' mechanism), iodination of thyroglobulin and release of thyroxine or triiodothyronine from the gland. Such a subdivision, although convenient, is somewhat arbitrary since it becomes increasingly clear that the metabolism of iodine must be considered within the thyroid gland as a whole.

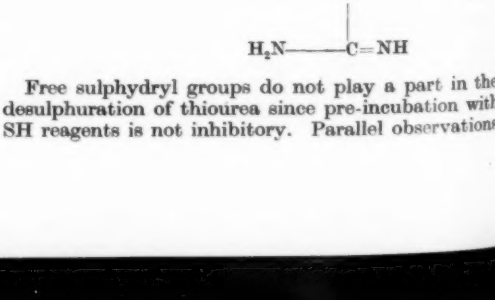
Thiocyanate and perchlorate are both known to prevent the accumulation of radioiodine by the thyroid. Wollman and Reed reported experiments designed to ascertain whether thiocyanate can, in addition, affect the iodination process and involving measurements of the binding-rate and thyroid/serum ratio (T/S) of injected radioiodide computed for different levels of serum thiocyanate. If two thyroidal compartments, an iodinating and a non-iodinating, respectively, were envisaged and the concentration of radioiodide in the latter was assumed to parallel that of serum, binding-rate constants became independent of serum thiocyanate-levels, consistent with the hypothesis that thiocyanate acts solely by decreasing the concentration of radioiodide at the binding site.

Perchlorate is excreted virtually unchanged (Lewitus *et al.*). Administration of $K^{36}Cl^{18}O_4$ showed that it is distributed in the body as is the iodine ion, being similarly concentrated in the thyroid gland. The supposed antagonism between perchlorate and iodide is of a competitive nature. These two ions have nearly the same ionic volume. Monofluorosulphonate, difluorophosphate and fluoroborate also have approximately the same ionic volume and are isoelectronic with perchlorate, and trial shows that they also inhibit the uptake of iodine by the thyroid. These studies lead to the conclusion that the 'trapping mechanism' may be merely a specifically adapted protein with a spatial arrangement that will chelate those ions which will fit into its spatial structure.

The synthesis of thyroglobulin in the mouse has been followed autoradiographically after injection of labelled leucine (Nadler *et al.*). Maximum activity was seen in the epithelium within 0·5 hr., followed by a decline as activity rose in the colloid (maximum in 35 hr.). These results are interpreted as indicating synthesis of a pre-thyroglobulin in the epithelium followed by secretion as thyroglobulin into the colloid, where it is iodinated prior to breakdown and release of thyroxine and amino-acids. The number of cells in the epithelium controls the rates of these processes. De Groot reported upon the early stages of *in vitro* hormonogenesis in sheep thyroid slices and in living rats. A homogenate of the thyroid tissue was separated into mitochondrial, microsomal and soluble fractions which were then analysed. Soluble fraction protein-bound iodine-131 as per cent of total protein-bound iodine-131 increased rapidly, then plateaued and was apparently all thyroglobulin. No thyroxine was identified in slice homogenates. It seems that in the iodination of thyroglobulin, iodine-131 may be transferred from a particulate fraction.

There is still much discussion concerning the possible role of a thyroid peroxidase in oxidizing iodide to iodine available for iodination. This chemical reaction can occur *in vitro* and therefore seems attractive, but it will be recalled that, among others, Glock[3] has severely criticized the evidence purporting to show the presence of a true peroxidase in the thyroid gland. Alexander reports that thyroid and salivary gland homogenates oxidize iodide in the presence of hydrogen peroxide added as such or generated by auto-oxidation of flavin. Catalase or anaerobiosis prevents flavin activation. The iodine formed by oxidation will iodinate tyrosine and to a lesser extent histidine, but the presumed natural acceptors, thyronine and di- and tri-iodothyronine, are *not* iodinated by this system.

That biological iodination may, in fact, be brought about by a different mechanism is suggested by a most interesting communication from Maloof and Soodak. These authors had previously demonstrated the desulphuration of sulphur-35-labelled thiourea by a cytoplasmic particulate system of thyroid. Thiouracil and thioacetamide are similarly desulphurated, the products being about 50 per cent protein-bound sulphur-35, 27 per cent thiosulphate, 10 per cent sulphate and 10 per cent an unknown compound. The protein-bound sulphur-35 is non-dialysable and stable to acid, but 80 per cent of it is displaced by such alkaline reagents as cyanide, sulphite, sodium hydroxide or by thiols such as cysteine, reduced glutathione and diethyldithiocarbamate. The desulphuration of thiourea is strongly inhibited by sulphite and cyanide, nucleophilic anions which are known to split disulphide bonds forming a thiol and an S-alkyl thiosulphate or alkyl thiocyanate, respectively. Using $Na_2{}^{35}SO_3$, it is found that it also forms protein-bound sulphur-35, which is quantitatively and qualitatively similar to that arising from labelled thiourea. The results suggest that both react with thyroid protein disulphide bonds forming a new mixed disulphide linkage as follows :



Free sulphydryl groups do not play a part in the desulphuration of thiourea since pre-incubation with SH reagents is not inhibitory. Parallel observations

on the iodination reaction in this *in vitro* system suggest that a disulphide bond may be involved in the formation of an active iodinating intermediate. Hence, cleavage of an S—S bond in thyroid tissue by thiourea may be a factor in the inhibitory action of this compound. The desulphurating effect appears to be specific since it did not occur with liver tissue preparations.

Anti-thyroid drugs are not only of great therapeutic value, but also the study of their action continues to throw light upon the mechanism of biosynthesis of thyroxine, as the last-mentioned contribution clearly shows. For the medical treatment of thyrotoxicosis in the United States, according to McClintock and Gassner, propylthiouracil, 1-methyl-2-mercaptoimidazole and 5-iodo-2-thiouracil are most commonly used, while the British 'Carbimazole' has only been introduced there during the past year. The trend is to use these drugs for pre-operative preparation although their use as the sole method of curative therapy still has adherents. The first three have been compared for involutional activity. 5-Iodo-2-thiouracil appeared superior and gave less trouble during subsequent surgery. All the compounds are, however, associated with toxic reactions (4–8 per cent). It may be mentioned in this connexion that one of the advantages of 'Carbimazole' is its comparatively low toxicity.

The mode of action of anti-thyroid drugs has been investigated by Rimington. Recent work shows that where thiol ⇌ thione tautomerism is possible, involving carbon and nitrogen, compounds exist mainly in the thione form (hydrogen on nitrogen not on sulphur); configuration may be 'fixed', however, by alkylation. Using 'Neo-mercazole' ('Carbimazole': 1-methyl-3-carbethoxy-2-thioglyoxalone) for standard reference, a series of compounds were compared including derivatives of 3-mercapto-1,2,4-triazole and the alkyl-fixed thiol and thione form corresponding to 'Neo-mercazole' itself, namely, 1-methyl-3-ethylglyoxaline-2-thione and 2-ethylmercapto-1-methylglyoxoline. None was as potent an anti-thyroid in rats as 'Neo-mercazole' and the thione configuration appeared to be that responsible for anti-thyroid action. Measurement of the inhibitory effect of these drugs upon a horseradish peroxidase system *in vitro* showed no correlation with anti-thyroid activity.

Mayberry and Astwood have used propylthiouracil to study the intrathyroidal metabolism of iodine in rats. The distribution of iodine-131 in controls 24 hr. after dosing was triiodothyronine 2·6 per cent, thyroxine 21·2 per cent, iodide 2·7 per cent, monoiodotyrosine 25·2 per cent, and diiodotyrosine 42·4 per cent, and these proportions varied little during six days while 53 per cent of the iodine-131 left the gland. In the propylthiouracil-treated group, 92 per cent of activity left the gland in the same time, while the proportion of thyroxine steadily diminished. If recycling of iodine-131 was prevented by a large dose of iodide following the isotope, rate of loss of iodine-131 was only slightly greater than in the controls and the distribution was similar except for a slow drop in monoiodotyrosine. These results suggest that propylthiouracil not only blocks iodination but may also inhibit coupling of iodotyrosines.

The lipid metabolism of the thyroid has been investigated using phosphorus-32. Experiments on rats of depletion of sialic acid, iodine-131 and iodine-127 from the gland indicate that the concentration of the former affords a measure of the thyroglobulin content. Histochemical methods have been used in investigations of prolonged dietary iodine deficiency and of normal and pathological glands. Lindsay and Jenks studied a number of dehydrogenases, diphosphopyridine nucleotide and triphosphopyridine nucleotide diaphorases, monoamine and cytochrome oxidases and found most of these enzymes increased in animals treated with propylthiouracil, but greatly decreased after hypophysectomy or thyroid feeding. Tricarboxylic acid cycle enzymes are present in thyroid glands, but in lower concentrations than in sheep liver (Dumont). They are not inhibited by anti-thyroid drugs and are raised in glands from treated thyrotoxicosis patients. Blood pyruvic acid is high in toxic goitre but normal in non-toxic goitre (Gasiorowski). It remained high after attainment of the euthyroid state.

The firmness of binding of thyroxine in sera has been measured by determining the proportion held back on a suitable resin; enhanced binding is found in myxœdema, after propylthiouracil and in pregnancy but weak binding in hyperthyroidism (Mitchell *et al.*). Attention was, however, directed by Hamolsky *et al.* to the effects of factors such as carbon dioxide tension, buffers and *p*H on thyroxine binding in sera. At *p*H 8·6 an irreversible increase in binding occurs. The pattern of thyroxine binding in normal and pathological conditions has been the subject of several investigations. The pre-albumin shows a greater elective affinity for triiodothyronine than for thyroxine (Andreoli *et al.*).

The discovery of triiodothyronine has turned attention to the deiodinating enzymes and the metabolism of the thyroid hormones. Thus, Lissitzky *et al.* reported that thyroxine bound to specific binding protein is protected from deiodination and that in cells there is competition for thyroxine between deiodinase and thyroxine bound protein.

The tadpole has long been used for the study of thyroxine. With its development into a frog, concomitant alterations occur in responsiveness to thyroid hormone and the manner or rate at which it is metabolized (Galton and Ingbar). The results presented are consistent with, but do not prove, the hypothesis that hormonal response is linked to hormonal degradation. The fate of the doubly labelled sulphuric ester of triiodothyronine in the rat is rapid hydrolysis followed by deiodination. It appears to be either a storage or transport form of the hormone (Roche *et al.*). Radioactive 'Triac' and 'Tetrac', the acetic acid analogues of triiodothyronine and thyroxine, respectively, are found by Green and Ingbar to be metabolized differently in man.

Peripheral biochemical anomalies as well as thyroidal disturbances are thought to be present in sporadic cretinism from studies by Klein of the half-value times of thyroxine metabolism.

The relationship of pituitary to thyroid function has been studied by Becker *et al.* Patients with active or inactive Graves's disease did not show an immediate decrease of thyroid function following total hypophysectomy.

One of the most difficult conditions commonly associated with thyrotoxicosis to understand, and also one of the most unpredictable in its course, is exophthalmos. A number of interesting papers were read on the subject. Bartels and Irie showed, by studying patients suffering from severe progressive exophthalmos, that there was no close relationship between the development of the condition and the functional state of the thyroid or the type of treatment given for hyperthyroidism. Hitherto the course, degree and severity of exophthalmos have often been

regarded as being influenced by the type and extent of the treatment offered for thyrotoxicity. A significant minority of patients developed exophthalmos before hyperthyroidism, and in five cases of severe exophthalmos no hyperthyroidism was noted at any time.

Dobyns described the use of fish (*Fundulus heteroclitus*) for the measurement of the level of an exophthalmos-producing substance in serum. Only in patients with active, progressive exophthalmos was exophthalmos-producing substance clearly demonstrable. Condliffe *et al.* provided evidence, from work with ion-exchange chromatography, that thyroid-stimulating hormone and an exophthalmos-producing substance are separate entities.

Contributions to our knowledge of the relationship between pituitary and thyroid activity, as well as a further understanding of those substances circulating in the blood stream which affect the activity of the thyroid gland, both in health and disease, were made by a number of authors. Two important papers (Purves and Adams, and J. M. McKenzie) described an abnormal thyroid-stimulating agent present in the blood of hyperthyroid persons. It appears not to be present in normal persons; the substance differs from normal thyrotrophin, for example, in the duration of its effect on the thyroid of guinea pigs and mice. It is active far longer than thyroid-stimulating hormone. The substance is active in hypophysectomized animals, thus demonstrating that it acts directly on the thyroid. The amount in serum appears to be correlated more closely with the degree of exophthalmos present than the severity of hyperthyroidism. Halmi *et al.* showed that thyroid-stimulating hormone stimulates rapidly the output of iodide from the thyroid, but the uptake of iodide more slowly.

El Kabir reports a reliable assay method for thyroid-stimulating hormone based on release of iodine-131 in incubated guinea pig thyroid slices. Generally speaking, thyroid-stimulating hormone values were high in patients with thyrotoxicosis and untreated primary myxœdema, whereas in exophthalmic ophthalmoplegia the values did not differ appreciably from normal.

Hellman *et al.* pointed out that apart from the differences in thyroxine-levels to be found in myxœdema and hyperthyroidism, there were also profound differences in the metabolism of androgens. If the thyroid dysfunction is corrected, disturbances in the androgens return to normal. Androsterone, of which low levels are found in hypothyroidism and high levels in hyperthyroidism, can be shown to produce to some extent the actions of thyroxine itself (thyromimetic effect).

No significant thyroxine formation was found by Taurog *et al.* in thyroidectomized rats. On the other hand, large daily injections of iodide had a stimulatory effect on growth. Since no thyroxine can be detected in the plasma, the possibility must be considered that iodide itself may have a thyroxine-like effect on tissues when given in high concentrations.

The connexion between serum cholesterol-levels, hyperthyroidism, hypothyroidism and the incidence of myocardial infarction has fascinated many, and a number of papers were devoted to these subjects.

One of the most interesting concepts which has arisen in recent years in connexion with the etiology of disease is that of auto-immunization, namely, the production by the body of antibodies against one of its own tissue components. That this could be a mechanism has often been suspected, but not clearly demonstrated, until Doniach and Roitt's work on Hashimoto's thyroiditis. Positive serological reactions can be obtained with the serum of such patients. Trotter and Belyavin observed complement fixation reactions between thyroid extracts and sera from patients of nearly all cases of Hashimoto's thyroiditis. The antigen is probably derived from the thyroid cells and appears to reside in the microsome fraction. Most cases of spontaneous myxœdema and about one-third of patients with thyrotoxicosis afford serum with a relatively low titre, as do occasional cases with non-toxic goitre, thyroid carcinoma or normal subjects. This cellular antigen can be detected in extracts of all types of thyroid, but is most abundant in thyrotoxic glands. Fixation of complement by the antigen and its corresponding antibody may be prevented by an inhibitory factor. The serum from patients with Hashimoto's disease commonly contains antibodies also against thyroglobulin. These are demonstrable by precipitation reactions and are distinct from those to the 'cellular antigen'.

A striking objective demonstration of the cytotoxicity of Hashimoto's sera was presented in a film by Pulvertaft *et al.* Primary cultures of human thyroid cells put up in fresh Hashimoto's serum showed few, if any, surviving cells at 18 hr. Substitution of Hashimoto's serum for normal serum in an 18-hr. old culture was followed by immediate darkening of the filamentous mitochondria and their gradual dissolution followed by rapid death of the cell. On the other hand, cultures grown for 36 hr. appeared to remain unaffected. The factor in Hashimoto's serum is thermolabile and specific; it does not kill all cells in a dense culture. Somewhat conflicting evidence was presented by Irvine, who argues that Hashimoto humoral antibodies are of secondary etiological importance. He considers that the basic pathology of Hashimoto's disease may be damage to the basement membrane, possibly due to cell-bound antibodies. Humoral antibodies, secondary to the escape of colloid, would then be an effect, rather than a cause, of the disease. However, since serological reactions are frequently demonstrable in thyroid disease, even in a small percentage of cases of unequivocal thyrotoxicosis according to Alexander *et al.*, and since some patients without Hashimoto's disease have a few clinical features of thyroid over-activity, screening tests for Hashimoto's thyroiditis should always be undertaken.

Two papers were presented in which the incidence of cancer was related to the use of radioactivity therapeutically. Winship and Rosvoll discussed an attempt which had been made over twelve years to examine the past history of children who, prior to the age of fifteen years, developed cancer of the thyroid. Where it has been possible to interrogate the parents of the children very fully, in 81 per cent of the cases there was a history of irradiation having been given therapeutically for a variety of reasons. Commonly, X-ray therapy had been given to try to treat an enlarged thymus. Sometimes similar treatment had been given for enlarged tonsils and adenoids; in others, for hæmangiomata and nævi. The latent period for the development of malignancy appears to be 7–12 years, and in most cases 400–700 r. had been given. The most common type of carcinoma encountered was the papillary type. Another investigation of a not dissimilar character was reported by Pochin. Careful analysis of the actual incidence of

leukæmia in patients treated with radioactive iodine for cancer of the thyroid, when compared with the incidence of the disease in the healthy population at large, suggests that leukæmia may have to be regarded as a calculated risk (even if only a small one) when this treatment is used for thyroid cancer. Pochin, however, suggested that when smaller dosages are given for the treatment of thyrotoxicosis, the present known incidence of leukæmia does not appear to be greater than that which could have been said to have arisen by chance. Nevertheless, it is clearly desirable to watch this situation carefully and to extend observation over a longer period.

Ackerman *et al.* reported a simple and accurate test for the diagnosis of thyroid cancer. This consists of giving orally 500 mc. of phosphorus-32. Twenty-four hours later, multiple counts are taken of the radioactivity from suspicious nodules. In six cases uptake of phosphorus-32 was increased markedly. These were afterwards shown to be malignant tumours. It was very interesting to find that one case of Hashimoto's disease also suggested malignancy by this test. Thirty-four negative tests were produced and in all those operated upon (22), except one, benign tumours were diagnosed.

It is twenty-two years since the last International Goitre Conference, and although striking advances in both knowledge and technique have occurred during this time, it will be apparent that many fundamental problems still remain unresolved.

The late Edwin Ramsdell had prepared an address which was read at the opening of the conference. In it he reminded the members that at the third conference there had been no mention even of anti-thyroid drugs, let alone of radioactive isotopes. How completely the position has changed will be evident from the wide compass of topics dealt with in 1960.

C. RIMINGTON
R. A. MILEY

[1] Astwood, E. B., *et al.*, *J. Biol. Chem.*, **181**, 121 (1949).
[2] Bachelard, H. S., and Trikojus, V. M., *Nature*, **185**, 80 (1960).
[3] Glock, G. E., *Nature*, **154**, 460 (1944).

THE BRITISH COMPUTER SOCIETY

HARROGATE MEETING

SOME 400 delegates attended the second annual conference of the British Computer Society, held at Harrogate during July 4–7. The president, Dr. M. V. Wilkes, noted with satisfaction that the Society, with one annual meeting behind it, was indeed flourishing.

In his opening address, Dr. Wilkes announced the formation of an International Federation of Information Processing Societies, in which the British Computer Society would represent the United Kingdom. The Federation has elected its secretary-treasurer (A. Speiser, Switzerland), president (I. Auerbach, United States) and vice-president (A. Walther, Germany), and has announced that its next conference will be in Munich in September 1962.

Dr. Wilkes referred to the activities of the publication committees of the British Computer Society, and especially to the technical committees on automatic programming. He thought that scientific auto-coding was now generally accepted, but that the break-through in auto-code language for commercial applications, the results of which could be devastating, was still to come. One of the most important current problems was that of data-transmission, and he compared the rather slow teleprinter circuit, with difficult checking facilities, with the telephone circuit with forward error-detecting codes and automatic repeat when necessary. An interesting point was that 100 per cent accuracy is not essential, the aim being to make the transmission of information as accurate as any further processing thereof. He envisaged the less-pecunious universities ringing round to their more fortunate brethren, sending their problems by telephone to the fastest and cheapest service. Again, however, he warned that the solution of the problem, though imminent, was not yet effected.

The second session discussed a common computer language. C. Strachey (formerly of the National Research Development Corporation) outlined the problems arising in the construction of such language and its advantages to programmers, and mentioned important advances made by the United States in the technique of 'list processing'. He made a plea for humans to regard their time as valuable, and to refrain from relapsing into slavery to the machine. R. Brooker (University of Manchester) spoke in technical terms of some recent work in constructing translating routines, carried out at Manchester, for any 'computer-oriented' language, and reported success with regard to the autocodes of *Pegasus* and *Mercury*. These methods apparently need a machine with a fairly large store, though K. Tocher (United Steel) reported success in devising restricted autocodes for smaller machines.

Business applications also have their auto language, and R. Paine (I.C.T.) outlined the desirable features of such a language and some attempts made to meet them. He warned that management cannot yet dispense with its programming staff, that the language of the accountant is not yet that of the computing machine, and envisaged the evolution of long-hand and short-hand versions of the language. This contribution included the comments that a computer conference might be described as a "meeting of contiguous word-noises", and that machine manufacturers' advertisements of business computer autocodes rarely err on the side of modesty.

At about this stage one notes with sorrow the transformation of certain features of the English language, and too often its mutilation which, in these vulgar times, seems to accompany the development of any new scientific field, and in which computer terminology is second to none. One finally applauded the question, "What exactly, please, is a programmer ?", and listened with regretful admiration to the chairman's "Since it is now time for coffee, I shall have to treat that question as rhetorical !"

The next session was also concerned with language and general-purpose programmes, this time for market research (A. Douglas, University of Leeds, and R. Cook, Elliott Bros.), which provided facilities

for receiving and verifying data, forming frequency tables, and printing results in any desired format, and in general statistical survey work (F. Yates, Rothamsted), and success was reported with several of the smaller machines.

Small computers were also featured in a symposium on their use in a variety of problems covering clerical processes, production-control in a medium-sized factory, an analysis of a reasonably complicated hydro-electric system, and stock control in a shoe factory. This sequence of thoughtful, well-presented papers seemed to leave the audience rather bemused. Did they, one wonders, ponder on the remarkable advances in the past decade when machines like the Stantec *Zebra*, ICT 1201, Elliott 402 and 802 and Ramac 305 can now properly be described as small, or were they shattered with the perfect simplicity of a data-transmission system in which a gamekeeper reads water-levels once a week, cycles ten miles, and sends off postcards ?

S. Clarke (Elliott Bros.) and S. Gill (Ferranti) gave accounts of recent respective visits to the U.S.S.R. and the United States. Clarke reported great Russian interest in British computing equipment and their own assessment of a four years lag behind the West. He described some Russian machines, none of which has a rapid access store of more than about 2,000 words, and noted that their present main interests were in automation and language translation. He found little effort in general data-processing and commercial applications other than in pay-roll work. The Chinese have apparently made a perfect copy of one of the largest Russian machines in one year, indicating perhaps the shape of things to come.

Gill found advances in the United States rather disappointing in actual machines, and thought that new British projects such as the *Atlas* have no clear trans-Atlantic superior. In programming techniques parallel operation and time-sharing are all the rage, but enthusiasm for international machine languages like *Algol* (scientific) and *Cobol* (commercial) are somewhat on the wane. For example, no *Algol* compiler is yet working efficiently for any machine in the United States, and university research is concentrated on more general studies in the theory of computer programming. He commented on the work of Hao Wang, an Oxford mathematical logician at present in America, who on an International Business Machines computer proved 350 significant theorems in "Principia Mathematica" in 8½ min., exclusive of printing time. Wang has remarked on the great significance for mathematics of the cross-fertilization of 'inferential calculus' and computing machines. Here is certainly a fascinating field of research, needing for its success mathematicians of the highest competence and machines of a size greater than any at present installed in a British university.

Attention was directed in one of the sessions to the organization of computing centres in universities (R. Buckingham, University of London) and industrial establishments (A. Bagshaw, Ferranti). University work covers a very broad field, mainly scientific, with consequent advantages for the 'open shop' method of working, in which most users write, test, and perhaps run their own problems. This, of course, is not always possible ; the 'unflappability' of the Chancellor of the University of Oxford would, one imagines, be tested severely, and unfairly, by his first few attempts at programme checking. Commercial centres have similar problems except that scientific work is beginning to be swamped by general data-processing calculations, with consequent greater economy in more operator-controlled machine running. This tendency will intensify when 'time-sharing' machines come into general use. A. Beaven (National Computer Research) had a different problem of organization, involved in the training of operators and the writing of programmes to check and correct data, derived from automatic paper-tape punches attached to accounting machines, for input to a computer, such data amounting to nearly three-quarters of a million paper-tape characters a month.

Time-sharing was put into practice when the numerical analysts, in surprisingly large numbers, discussed some new techniques for applying predictor-corrector formulæ in initial-value problems and for the iterative solution of non-linear boundary-value problems in ordinary differential equations, the determination of latent roots of matrixes when the parameter appears in non-linear form, and the behaviour of the remainder term and other aspects of Gauss-type quadrature formulæ. These papers comprised a small but fairly representative sample of current research in 'computer-oriented' numerical analysis. At the same time, the larger group studied problems of simulation in chemical plants, power stations and other works, the computer being used to make adjustments to the detailed process, using a 'mathematical model' and a statement of objectives, in order to increase efficiency and output.

On the last morning, D. Polley (Ministry of Pensions and National Insurance) and Col. L. Slater (Royal Army Pay Corps) gave accounts of respective progress in the installation of data-processing schemes for insurance records and for the payment of the military. In both cases the machinery required seemed to be substantial, with reels of magnetic tape and a 'Xeronic' printer on one hand, to cope with the processing, in a six-monthly period of each year, of thirty million tax-reduction cards, and on the other an International Business Machine 705 computer, 1,401 tabulation system and a staff of 100. Soldiers must have their pay, and one sympathizes with the problem of maintaining continuity in reimbursement and record in the process of changing from manual to automatic operation.

Finally, a small symposium (which might well have shared time with numerical analysis so that all, including myself, could have heard about simulation) discussed techniques of accounting, in wholesale and retail branch control, in 'invoicing' in general, in the paper industry in particular, and in banking. Here, it seems, the problems of programming are trivial compared with those of deciding the factors which should be taken into account (as, for example, the loss of weight in stored cheese), and of collecting all the parts into a coherent logical entity.

The organization of the meeting was good and its after-effects promising, since those who could not attend will apparently have a chance to study at more leisure at least some of the main contributions, which will be published in the *Computer Journal*. We lacked only sunshine (perhaps mercifully in the Harrogate Sun Pavilion) and, as usual, time for adequate discussion. The Pavilion was just large enough to accommodate the full audience and reception and entertainment, provided by the Mayor, and a small exhibition of computing mechanisms, models and plans, provided by the leading British manufacturers, in an atmosphere at once both sufficiently formal and intimate.

L. Fox

THE MUSEUMS ASSOCIATION

THE sixty-sixth annual conference of the Museums Association was held in the city of Stoke-on-Trent during July 4–8, by kind invitation of the Corporation and University College of North Staffordshire. Dr. W. E. Swinton, of the British Museum (Natural History), presided over some 330 delegates.

The conference was warmly welcomed at this its first visit to Stoke by the Lord Mayor, and in his presidential address Dr. Swinton dwelt on the present plight and difficulties of the museum movement. He welcomed increased grants made by the Treasury to the national museums, and the provincial museums through the scheme administered by the Victoria and Albert Museum. He also looked forward to the founding of further regional councils based on the existing prototype in the south-west of England. Dr. Swinton deplored the absence of travelling museums in rural districts, for they had been so eminently successful in the United States, and he felt convinced that they would supply a real need in Britain. Dr. Swinton, taking his text from the underground geology in the area, then discussed the injection of art and science into industry and the way in which literature and the arts had influenced the industrial life of the city.

The presidential address thus paved the way for the main discussion of the conference, which concerned itself with the relations of museums and industry, introduced by Sir Colin Anderson, Mr. Ian Cox, Mr. John Moores and Sir John Wedgwood. All speakers agreed that with the competition of television and shop displays the standard of exhibition in museums should be high and modern in outlook. It was also felt that the displays should be stimulating and give pleasure as well as instruction. Curators should study the methods of salesmanship. In the same way that no shop would freeze its windows, museums must be prepared to change their displays as often as possible. Museums, on their part, can do much to stimulate new designs and help to maintain high standards of craftsmanship.

Another important discussion concerned the purchase of art objects for museums. The speakers included Sir Anthony Blunt, Dr. Trenchard Cox, and Messrs. G. L. Conran, Terence Mullaly and Hugh Scrutton. It was generally agreed that directors should make more frequent contact with the London market, although even then purchases were to a great extent a matter of chance with either local or national opportunities. It was also stressed that rare and expensive objects should not be the desiderata of museums but they should endeavour to create an individual character.

At the annual general meeting it was decided to increase institutional members on the Council from three to six and to request nominations for these from regional organizations.

Dr. D. B. Harden (London Museum) was elected president and Mr. Phillip James was confirmed in his office as secretary. It was decided to hold the next conference at Plymouth during June 19–24, 1961.

Social functions included an informal reception at Keele Park by invitation of the University College, a reception and dance by invitation of the Lord Mayor and tours of the Arnold Bennett country, the Potteries, Kedleston and Dovedale. Official visits were also made to the local museums.

THE BRITISH STEEL CASTINGS RESEARCH ASSOCIATION

THE progressive growth of one of the youngest research associations was marked recently by the open days held during June 28–30 by the British Steel Castings Research Association at its Research Station in Sheffield. The open days were attended by 546 representatives, principally from member companies and from users of steel castings.

The opening of the Association's Research Station was noted in these columns (*Nature*, **180**, 221 ; 1957). Within three years the Association has found it necessary to add a further 10,000 sq. ft. to its accommodation, while its staff has increased in this period from forty-nine to seventy-six. The increased accommodation has provided substantially more space in laboratories devoted to chemical analysis, metallurgy, sand testing and mechanical testing. The machine shop has been correspondingly enlarged, and new laboratories have been provided for radiography and the handling of isotopes used in investigations employing radioactive tracers and for the analysis of gases in steel. The experimental foundry has also been enlarged to the extent of 50 per cent in floor area, providing floor space for the installation of a 56-lb. vacuum melting furnace, for pilot plant studies of dust and fume collection equipment and for the study of foundry plant and equipment on a scale approximating to that of installations in production foundries. The total cost of the extensions together with the equipment installed therein has been £60,000, bringing the total cost of the Research Station to £165,000 within a period of four years. The present income of the Association is a little more than £100,000 per annum. The number of steel foundries in Great Britain is not large, and the present subscription income represents an average subscription of £1,000 a member. That the Association can maintain such a high level of subscription and has, in fact, recently increased the subscription rate by nearly 10 per cent is indicative of the importance that the industry attaches to its co-operative research.

The demonstrations of the Association's work, which were on show at the open days, illustrated very well the point that many of the problems associated with the production of steel castings are non-metallurgical in character. A large proportion of the research effort is concerned with moulding materials and with the interactions that occur at high temperatures between molten steel and the sands and

other refractories from which moulds are made. One of the Association's major problems has been to evolve quantitative techniques for the measurement of such phenomena as the friability of moulding sands on drying, the erosion of moulds that occurs when molten steel flows over the surface, the penetration of molten steel into the interstices between sand grains and the 'flowability' of moulding sands, that is, the ease or difficulty with which they may be compacted in narrow pockets, etc. Techniques have been evolved for these and similar measurements; for example, it has been possible, by using a test casting in which a controlled air pressure is applied to the liquid steel, to synthesize in a casting only 15 in. high the ferrostatic head experienced at the bottom of a casting 20 ft. in height. This technique has greatly facilitated the study of the penetration of liquid steel into moulding materials.

Much attention has been given to the development of mould paints and washes, and new formulations based on the dispersion of refractory particles in alginate suspensions have been developed which produce paint films that do not crack on drying or when molten steel is poured into the mould. These are a considerable advance on commercially available washes and are being applied on an increasing scale in the foundries of the Association's members.

The engineering section of the Association devotes considerable effort to studies of plant and equipment used in foundries and is actively engaged on investigations of factors affecting the performance of moulding machines and shot-blasting equipment. Studies are also being made of both wet and dry methods for the reclamation of used foundry sand. Here the problem is to remove from sand grains the layer of dead bonding clay to enable the sand to be re-bonded for cores and moulds.

From its inception the Association has always maintained a lively interest in problems relating to industrial health in foundries. Its recent activities have included the development of an exhausted fettling bench which combines control of fine dust with a substantial reduction in noise pressure-level when castings are cleaned with pneumatic chipping hammers. These benches are now being made by two manufacturers.

Studies are also being made of atmospheric pollution from foundry steel-making furnaces, and a survey has recently been completed of the fume emission of arc furnaces varying from 30 cwt. to 80 tons capacity and from side blown ('Tropenas') converters.

The Association attaches considerable importance to liaison with its member companies, and they are regularly visited by senior members of the staff to discuss aspects of the Association's work ripe for development and also to advise on technical problems arising in production. Conferences and discussion groups are also organized both on a national and a regional basis, and during the past year an average of eight representatives per member company participated in these meetings. The Association also organizes short training courses for representatives of members on subjects such as moulding sand control, steelmaking techniques, etc. During 1961 a training course is to be organized on work simplification in foundries.

The seventh annual report of the Association has recently been published, and in presenting this to the annual general meeting on June 28, Dr. R. Hunter (Clyde Alloy Steel Co., Ltd.), the chairman of the Association's Council, invited members' attention to notable features of the report. He directed special attention to the expanded programme of liaison with industry referred to above and also to the establishment of a joint committee, the Steel Castings Development Committee, between the Research Association and the trade association, the British Steel Founders' Association. The function of this Committee is to disseminate data designed to promote the greater use of steel castings, and the Research Association has in hand a substantial programme of work for this Committee to determine properties of steel castings on which data are now lacking. A useful start has also been made in the provision of lectures and lecture material for presentation to engineering societies and engineering departments of technical colleges.

A. H. Sully

CULTURE, SOCIETY AND HEALTH

During June 1–3, 1960, the Division of Anthropology of the New York Academy of Sciences and the Research Institute for the Study of Man jointly arranged a conference on culture, society and health. Among the many interesting papers was one dealing with cancer frequency and socio-cultural factors and another which examined the frequency of coronary artery disease in population groups. The study of socio-cultural factors in cancer was carried out by Walter B. Quisenberry, Division of Preventative Medicine, State Department of Health, Honolulu.

The population of Hawaii is made up of approximately 35 per cent Japanese, 23 per cent Caucasians, 18 per cent Hawaiians and part-Hawaiians, 12 per cent Filipinos, 6 per cent Chinese and 6 per cent others such as Koreans, Samoans, and Puerto Ricans.

Quisenberry's investigations showed that the incidence of stomach cancer is highest in Japanese men. There are probably many socio-cultural factors such as dietary habits which play a part in causing this. Primary cancer of the liver, however, is most frequent in Filipino men. Filipino men probably eat foods which are higher in carbohydrates and lower in protein and vitamin B_1 than those of other ethnic groups.

Filipino and Japanese men have the lowest incidence of lung cancer in Hawaii; Japanese men and Filipino men have probably smoked fewer cigarettes over the years than Caucasian men. The Caucasian breast cancer rate is five times the Japanese rate; Japanese women have been slower in giving up the nursing of children than have the Caucasians. Cancer of the large intestine is more frequent in Caucasian women than in any other ethnic groups; the diet habits and, perhaps, the bowel habits of Caucasian and Japanese women are different.

Cancer of the prostate gland is about nine times more frequent in Caucasian men than in Japanese men; the socio-cultural factors which may be responsible for this great variance in incidence of prostatic cancer may be related to differences in love-

making and mating habits of the two ethnic groups. Cancer of the nasopharynx is most frequent in Chinese; it is possible that there is irritation of the nasopharynx in Chinese from hot liquids such as tea.

Cancer of the uterine cervix is most frequent among Hawaiian and part-Hawaiian women; because Hawaiians are by nature phlegmatic, the women probably do not receive as good care following pregnancies as do women of other ethnic groups. Skin cancer is most frequent among Caucasians; this is due probably more to differences in skin pigmentation than to socio-cultural factors.

Quisenberry suggests that some of the ethnic differences in the frequency of cancer may disappear with the integration of different racial groups in Hawaii. The changes may bring about either lower or higher incidences of cancer. For example, cancer of the breast seems to be increasing in Japanese women due to the stopping of nursing of children.

The study of coronary artery disease was carried out by David M. Spain of Beth El Hospital, New York, and indicates that there has been a significant increase in the incidence of coronary atherosclerotic heart disease over the past twenty-five years in the United States. The possible environmental factors associated with this increase have been evaluated in relation to the possible common denominators in the environment believed to be associated with the reported low incidence of coronary artery disease in the South African Bantu, the Eskimo and the Chinese.

The preliminary findings of factors thought to be associated with coronary atherosclerotic heart disease in a study on several thousand males of the same ethnic origins from the New York metropolitan area were presented to illustrate the complex problems involved in the validation of the significance of such data. Such questions as standards of measurement, criteria for diagnosis, body build, occupational physical activity, obesity, hypertension, serum lipids, urinary œstrogens, diet and smoking habits were analysed while the limitations of studying coronary artery disease in living population groups, as well as the fallacy of generalizing from the findings in autopsy populations, were clearly shown.

THE STRENGTH OF SOLIDS

PROF. A. H. COTTRELL in his discourse to the Royal Institution on June 15, entitled "The Strength of Solids", emphasized the importance of this subject in engineering, and also in the functioning of the bones and muscles of the human machine he distinguished between two kinds of strength—that limited by plastic yielding and that limited by fracture. The manner in which a material fails depends on the relative values of strength against such ductile or brittle failure.

Those brittle materials which have very high inter-atomic forces and which should therefore have very high strengths are nevertheless weak due to the presence of small cracks or notches which act as stress concentrations. When a relatively flawless specimen of such a material can be prepared, it is found to have a strength that approaches the theoretical value. It is not, however, practicable to make such specimens in large enough size or sufficient quantity to be of use for structural components in engineering.

The ultimate strength of a ductile material depends on the stress at which a neck begins to form in a tensile test, that is to say, on its plastic behaviour, which involves the yield stress and work-hardening characteristics. An over-hardened material will not be ductile. The plastic deformation of crystalline solids is due to the sliding of crystal layers over one another. Slip over any layer is not simultaneous, but is due to the movement of a dislocation line which separates the slipped from the unslipped portion. Ordinary solids in engineering contain many sources of dislocations so that they are intrinsically soft and ductile. The vast amount of plastic deformation permissible enables a ductile material to absorb the energy of shock loading and is a protection against fracture. Strengthening is achieved by placing obstacles in the paths of the dislocations—by alloying, heat treatment, neutron bombardment, etc. If, however, too much strengthening is given the plastic yielding strength may exceed the fracture strength and the material would become brittle.

Steel, though normally ductile, is a borderline case, low-temperatures or impact loading or a combination of these factors can cause brittle failure—a phenomenon which has led to many engineering failures.

Prof. Cottrell concluded with some remarks on fatigue failure, which is not yet fully understood. It is observed that in fatigue, plastic deformation becomes concentrated on a few slip planes, so that slip bands develop and then initiate cracks. It would appear that this is a process of local work softening.

K. J. PASCOE

RESEARCH IN CASE STUDIES

THE use of case studies in management training is well acknowledged as an important method of linking practical problems with management theory. However, as a training technique it is still unfamiliar to many people; and many who have heard about it have had no experience of it. Such was the basis and cause of a proposal by the South London Committee during the 1959–60 session to explore the methods of using case studies. This promised to bring significant benefits to members of the British Institute of Management in the South London area.

The effective use of a case-study discussion demands considerable skill on the part of the chairman if it is to have a real impact on every member of a case-study discussion group, from the vociferous to the reticent, and worth-while contributions from all are to be obtained. On account of this, the Committee felt that it should obtain guidance and training from an authoritative source. Co-operation was therefore established with Miss Avice V. Turnbull, research officer on case studies, Royal Institute of Public Administration, and special lecturer in management

studies, Northampton College of Advanced Technology, London, and Mr. Z. M. T. Tarkowski, adviser on training, and special lecturer in management studies, Northampton College of Advanced Technology, London, who have done a considerable amount of work in this field.

The South London Committee convened a Case Study Group, at the meetings of which, at monthly intervals, various case studies were considered. The bulk of those so far discussed were from a series produced by Mr. Tarkowski, which give a representative sample of management situations, each in a typical British context. The way discussion went was analysed afterwards, under the leadership of Miss Turnbull.

The wisdom of selecting this approach became apparent early on in the series of meetings of the Group. Postmortems brought out the many interrelated points which have to be recognized and brought to bear on each other by the chairman. The effect of the size of the group, of a time limit, of the extent of the previous experience of the use of case studies, the attitudes of members, the need for the leader to draw members into the discussion and to encourage cross-fertilization of ideas without imposing on the group, are some of the many points which were discussed and which call for skilful leadership.

The reaction of the group depended upon the nature of the case study. If, at first sight, a specific management problem was apparent, or if the case study described a dramatic situation, such as a conflict of actual decisions taken, there was a tendency to examine the obvious problem and to suggest a satisfactory line of action which could be the next step to be taken in the situation. Where the management problems were more intangible and demanded a deeper analysis of individual characters, and of the situation, the more limited approach was replaced by a broader consideration of the personalities and the influences of the background in the work situation which caused a strained atmosphere. In fact, more than one problem can be isolated in these case studies, depending on the perception of individual members of a group. Practice in this type of analysis gave more satisfaction to the members of the Group. At the last meeting held, a case study already previously discussed was re-examined, and comparisons were made with the first discussion. These brought out the wide range of issues which can be illustrated by a single case study, and how diverse two discussions of the same case study can be.

Co-operating with Mr. Tarkowski and Miss Turnbull, several members of the Group have appeared as 'characters' in case study exercises held as part of a course at the Northampton College of Advanced Technology. The object of these exercises is to make the case studies more vivid and realistic, to bring out subtle issues of verbal communication and to give members of the group practice and experience in day-to-day interviews and conversations, which form a large proportion of a manager's work. So far as is known, no course on quite similar lines has been developed elsewhere.

The South London Committee has now arranged a week-end conference on "Research Necessary for the Preparation and Use of Case Studies: Some Recent Work and Prospects for Further Developments in Great Britain", to be held during October 7–9 at the British European Airways Staff College, East Burnham, Slough, Bucks. Further information can be obtained from A. E. Hoult, South London Branch, British Institute of Management, c/o Management House, Fetter Lane, London, E.C.2.

THE RESEARCH COUNCIL OF ALBERTA

THE fortieth annual report of the Research Council of Alberta, covering the year 1959, contains lists of advisory committees and their membership and of the staff of the Research Council besides a list of publications for the period 1955–59 (pp. v+54. Report No. 79. Edmonton: Research Council of Alberta, 1960). During the year, programmes on geology, ground-water and soils were brought together in an "Earth Sciences Branch", and work on coal, petroleum, natural gas and gasoline and oil testing in a "Fuels Branch". The ground-water studies are providing basic information on the ground-water resources of Alberta, and have offered substantial annual savings of water costs, for example, from the research on charging of water from Driedmeat Lake into a nearby gravel terrace. In surface geological mapping in the Taber area a buried Indian camp site was uncovered in the valley walls of the Oldman River, and along with Indian artefacts the skeleton of a buffalo and a willow branch were found, for which radiocarbon dating gave an age of 11,000 years. The Geological Division has developed a mineral dressing laboratory with the special purpose of investigating the possibility of refining Alberta sands. Studies of Cretaceous stratigraphy have been expanded into a co-ordinated programme, and a sub-surface study of Lower Cretaceous strata of central Alberta is in progress with the purpose of establishing the interrelations of the marine and non-marine zonations. Upper Cretaceous beds in the Peace River region have been zoned micro-faunally, and the southward marine extension of these strata is being examined for micro- and macro-faunal content.

The Coal Division is continuing an investigation of carbonization, which during the year has shown that the carbonization reaction is much faster than was thought, and the overall reaction is only slow because the coal-tar products have difficulty in working their way out of the carbonaceous residue; the overall reaction can be accelerated considerably in presence of nitric oxide. The Natural Gas Section is examining a process for recovering helium from natural gas, and the Petroleum Division discovered that mud from the bottom of a local lake contained completely developed petroleum pigments along with traces of other oil materials. Pilot plant experiments were extended to work on a concentric flow of heavy oil and water mixtures in a 1,000-ft. experimental pipe-line, which showed that injection of about 30 per cent of water gave a pressure drop of up to about 75 per cent. The Industrial Engineering Services Group has made good progress in studies on carburation to permit the satisfactory use of propane and butane as engine fuels. Studies have been commenced on the effects of various solvents on plastic

pipes, and a 500-ft. length of buried pipe carrying natural gas is now being observed under actual operating conditions.

Satisfactory progress is also reported in the highway research programme. The co-operative cloud physics and hail research programme is now in its fourth year, and the results should indicate whether Alberta hail is generally similar to that studied in Europe or whether there are significant differences in structure. The special weather-radar continues to give much information on the cloud systems moving through the area, and a meso-network of some thirty microbarographs has now been arranged at intervals of about 10 miles. Preliminary results from the study of nucleation represent a major advance in the basic approach to the hail problem, and following the study of nucleation commenced last year at McGill University, the work was continued during the summer with the project group at Penhold, where a method of determining the amount of 'freezing nuclei' in rain-water, or water obtained from melted hailstones, was applied.

ROTHAMSTED EXPERIMENTAL STATION

F. C. BAWDEN, whose style of writing could profitably be copied by directors of other research institutes, states in his annual report for 1959 of the Rothamsted Experimental Station* that the ultimate aim of agricultural research must be to increase the efficiency of agriculture by lowering unit costs of production and improving the quality of the produce. The very wide range of activities described in this report offers scope for assessing the contribution of Rothamsted towards this goal. Whether this aim will be better served by the empirical approach of much of the work reported, or by the establishment of general principles formulated on research of a more fundamental nature, is obviously a question which must exercise the mind of any good scientific leader. Perhaps the best compromise is to encourage developments along both lines, the correct allocation of resources depending on many factors.

There is no question that the work on the use of fertilizers, irrigation, and the control of weeds, pests and diseases has the immediate practical aim of increasing agricultural efficiency; but there is plenty of evidence throughout this report of more academic inquiries covering such varied matters as the underground activities of organisms as diverse as slugs, earthworms, fungi, nitrogen-fixing bacteria and an eel-worm that transmits a soil-borne virus; the internal structure of viruses and the relative infectivity of intact virus particles and their separated nucleic acids; the activities of enzymes in insects, bacteria and leaves; the biology of weeds and the relation between the potassium supply of soils and the nature of the clay minerals.

In the Chemistry Department, where work on fertilizers has always loomed large and has been responsible in no small measure for the present high efficiency of arable crop production in Great Britain, much basic work is now proceeding on soil organic matter, nitrogen, phosphorus and potassium. It might fairly be said that soil fertility studies at Rothamsted have suffered in the past from the 'NPK' (nitrogen–phosphorus–potassium) complex; but attention is now being directed towards other elements such as magnesium and molybdenum. Judged by the increasing incidence of hypomagnesæmia in farm animals, this interest is timely or even late, and if the work on micronutrients merely indicates that deficiencies of such elements are likely to be rare in Britain, it will serve its purpose.

Biochemistry increasingly emerges as the central discipline in agricultural research; and its impact can be seen in many departments ranging through soil chemistry, botany, pathology, insecticides and fungicides. In the Biochemistry Department itself, work is concentrated on plant enzyme systems, but there is some collaborative work with the Nematology Department on eel-worm hatching factors. One wonders whether the work on the extraction of protein from leaves has not now served its valuable purpose and should be handed over for large-scale development to industry and governments in countries where malnutrition is still a serious problem.

Russian claims that inoculation of soil with *Azotobacter* increases crop yields have been treated with scepticism in the Western world. None too soon, these reports are being properly investigated by the Soil Microbiology Department, and under some conditions, at present ill-defined, inoculation has increased yields significantly.

One of the dangers when a research station reaches the size of Rothamsted is that each department may work on isolated problems when more progress is likely to be made, particularly in biological research, by inter-departmental co-operation. There is disappointingly little evidence of this collaboration where it might be most valuable, for example, between the Chemistry Department, Pedology Department and the Soil Survey of England and Wales. That such co-operation is possible and fruitful is evidenced by the work of the Macaulay Institute in Scotland. On the other hand, it is pleasing to see the Botany Department is studying the effect of soil-water deficit on plant growth, clearly complementing the work in soil physics, where the study of agricultural meteorology, which has already had a notable effect on the practice of irrigation, is being extended into the field of micro-meteorology.

There is an encouraging sign of collaboration in the report of the Entomology Department. Changes in soil fauna brought about by long-term bare-fallowing are being studied, and at the same time other departments will study changes in soil organic matter, microflora, physical properties, organisms causing crop diseases, nematode populations, and the survival of weed seeds. Perhaps this will be the forerunner of other co-operative work, and for a start one might suggest similar studies when land is put down to grass. After all, grass is still the most important crop of Britain, and unless the Agricultural Research Council objects to duplication of effort, it would be stimulating to see Rothamsted paying more attention to grassland soils and some of the problems of herbage production.

T. W. WALKER

*Lawes Agricultural Trust. Report of the Rothamsted Experimental Station for 1959. Pp. 288. (Harpenden: Rothamsted Experimental Station, 1960.) 10s.

CONVERSION OF CYCLOPENTADIENE INTO NEW BICYCLOPENTYL DERIVATIVES

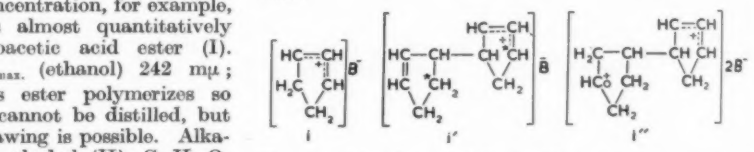
Structure Determination with the Help of Proton Magnetic Resonance Spectroscopy

Diels–Alder diene associations, including the dimerization of cyclopentadiene, are susceptible to trichloroacetic acid catalysis[1], with carbonium ions as intermediates[2]. The catalysed formation of endo-dicyclopentadiene, $C_{10}H_{12}$, was studied at acid concentrations up to 0·16 *M* (25°). It has now been found that at higher acid concentration, for example, 0·5 *M*, cyclopentadiene is almost quantitatively converted into a trichloroacetic acid ester (I). $C_{12}H_{13}O_2Cl_3$, n_D^{25} 1·5085, $\lambda_{max.}$ (ethanol) 242 mμ; $\varepsilon_{max.}$ 800 l./mol.-cm. This ester polymerizes so quickly on heating that it cannot be distilled, but purification by fractional thawing is possible. Alkaline hydrolysis produces an alcohol (II), $C_{10}H_{14}O$, b.p. 84–86°/0·2 mm., n_D^{25} 1·5105, $\lambda_{max.}$ (ethanol) 243 mμ, $\varepsilon_{max.}$ 400 l./mol.-cm., 3,5-dinitrobenzoyl ester m.p. 112–113°, phenyl urethane m.p. 64–65°. Bromination and hydrogenation established the presence of two double bonds in ester (I) and alcohol (II); the double bonds must be conjugated because of the specified position of the $\lambda_{max.}$ values.

There is no carbonyl frequency in the infra-red adsorption spectrum of alcohol (II), which excludes the possibility of hydroxyl being attached to unsaturated carbon. Hydrogenation of alcohol (II) produced a saturated alcohol (III), $C_{10}H_{18}O$, b.p. 95°/0·5 mm., n_D^{25} 1·4844, 3,5-dinitrobenzoyl ester m.p. 79–82°, phenyl urethane m.p. 88–92°, acid phthalate m.p. 80–82°, which was dehydrated to hydrocarbons $C_{10}H_{16}$, b.p. 112–115°/100 mm., n_D^{25} 1·4796, the infra-red absorption spectrum of which was very similar to that of a mixture of cyclopentyl–cyclopentenes, prepared according to v. Braun and collaborators[3].

Hydrogenation of both hydrocarbons gave bicyclopentyl $C_{10}H_{18}$, which proves that the alcohols (III) and (II) and ester (I) are bicyclopentyl derivatives. Alcohol (III) was oxidized to a ketone, $C_{10}H_{16}O$, b.p. 74–75°/1·3 mm., n_D^{25} 1·4743, 2,4-dinitrophenylhydrazone m.p. 129–131°, semicarbazone m.p. 197–198°, which was not identical with the well-known 2-cyclopentyl-cyclopentanone. Thus, the hydroxy-group of alcohols (III) or (II) cannot be attached to a carbon atom in juxtaposition to the junction points of the two five-membered rings. The proton magnetic resonance spectrum of pure liquid alcohol (II) is characterized by peaks *A*, *B*, *C* and *D* at − 0·82, − 0·05, 0·18 and 2·72 parts per million (p.p.m.). These chemical shifts (which are defined by the dimensionless number $[H_s - H_r/H_r] \times 10^6$ when H_s and H_r are the resonance fields of sample and reference (water)) are assigned as follows: *A* corresponds to hydrogen on unsaturated carbon, *B* to hydroxyl hydrogen, *C* to hydrogen bound together with hydroxyl to saturated carbon and *D* to the other hydrogens on saturated carbon. The assignment is consistent with the proton magnetic resonance spectra of alcohol (III) and cyclopentanol and with the relative peak positions in the pure liquid state and in carbon tetrachloride solution. Peak area measurements showed that four hydrogens must be attached to unsaturated carbon. An investigation of the proton magnetic resonance spectra of ester (I) and of the trichloroacetic acid esters of alcohol (III), cyclopentanol and ethanol lead to similar conclusions.

All the observations taken together prove that alcohol (II) is 3-(cyclopenta-2′,4′-dienyl)-cyclopentanol.



i *i′* *i″*

The new bicyclopentyl derivatives can be converted thermally and catalytically into polymers of unusual properties, which will be described elsewhere.

C. F. Blakely
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L. Roubinek
A. Wassermann
R. F. M. White

[1] Wassermann, *J. Chem. Soc.*, 618 (1942).
[2] Rubin, Steiner and Wassermann, *J. Chem. Soc.*, 3046 (1949).
[3] v. Braun, Kamp and Kopp, *Ber.*, **70**, 1756 (1937). Vapour phase chromatography showed that the 'cyclopentyl–cyclopentene' of these authors is a mixture of isomers.

Kinetics and Mechanism

The rate, R_{ester}, of the trichloroacetic-catalysed reaction, 2-cyclopentadiene + trichloroacetic acid → trichloroacetic acid ester of 3-(cyclo-2′,4′-dienyl)-cyclopentanol (see previous communication), (ester (I)), has been measured. The results can be summarized by:

$$R_{ester} = k_{ester}[\text{cyclopentadiene}]\,[\text{trichloroacetic acid}]^2 \qquad (1)$$

where k_{ester} is $10^{4\cdot3}\exp(-9900/RT)$(l./mole)^2 sec.$^{-1}$ in carbon tetrachloride and $10^{4\cdot4}\exp(-9700/RT)$ (l./mole)^2 sec.$^{-1}$ in benzene. It has been shown before[1] that the rate, R_{dimer}, of the trichloroacetic acid catalysed dimerization, 2 cyclopentadiene → endo-dicyclopentadiene, is given by:

$$R_{dimer} = k_{dimer}[\text{cyclopentadiene}][\text{trichloroacetic acid}] \qquad (2)$$

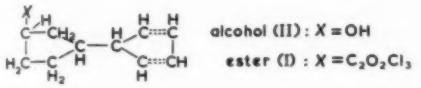
Using k_{ester} and k_{dimer}, relating to 25° and carbon tetrachloride, the ratios R_{dimer}/R_{ester} are 4·4 ± 0·8 and 0·022 ± 0·004 according to whether the trichloroacetic acid concentration is 0·0050 or 1·0 *M*. It is understandable, therefore, why the preparative isolation of ester (I) is only possible if the trichloroacetic acid concentration is sufficiently high (see previous communication).

Rate expression (1), the influence of polar aprotic solvents (nitrobenzene and nitromethane), which increase R_{ester}, and of proton acceptors (water, ethanol, acetone dioxan) which act as retarders, are compatible with a mechanism represented by:

$$M + HB \underset{k'_1}{\overset{k_1}{\rightleftharpoons}} i;\ i + M \underset{k'_2}{\overset{k_2}{\rightleftharpoons}} i';\ i' \underset{k'_3}{\overset{k_3}{\rightleftharpoons}} \text{endo-dicyclopentadiene}$$

$$i' + HB \underset{k'_4}{\overset{k_4}{\rightleftharpoons}} i'';\ i'' \underset{k'_5}{\overset{k_5}{\rightleftharpoons}} \text{ester (I)} + HB$$

In these equations M is cyclopentadiene, HB is trichloroacetic acid, i and i' are singly charged ion pairs $\overset{+}{C}_5H_7 \ldots \overline{B}$ and $\overset{+}{C}_{10}H_{13} \ldots \overline{B}$ and i'' is a doubly charged ion pair $\overset{+}{C}_{10}H_{14} \ldots 2\overline{B}$ (B = trichloroacetate). Application of the stationary state approximation and assuming that $k_1M[HB] \gg k'_2i'$; $k_2iM \gg k'_4i'' + k'_3$ [dimer]; $k_3 \gg k'_2$ or $k_4[HB]$; $k_4i' \gg k'_5$[ester (I)]; $k_2M \gg k'_1$ leads to (1) with $k_{ester} = k_1k_4k_5/(k_4' + k_5)k_3$ and to (2) with $k_{dimer} = k_1$. We suggest the following formulæ for i, i' and i''.



Conversion of i' into endo-dicyclopentadiene and of i'' into ester (I) involves removal of one HB; in the former case a C–C bond is formed between the carbon atoms indicated by asterisks, while in the latter case combination of one B^- occurs with the carbon atom indicated by a circle.

It has been shown (unpublished experiments) that the interaction of endo-dicyclopentadiene and trichloroacetic acid leads predominantly to the trichloroacetic acid ester (II′) of exo-hydroxydihydrodicyclopentadiene, rather than to ester (I). Thus the velocity coefficient, k_6, of the reaction endo-dicyclopentadiene + trichloroacetic acid $\xrightarrow{k_6}$ ester (II′) is probably large compared to k'_3.

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[1] Rubin, Steiner and Wassermann, *J. Chem. Soc.*, 3046 (1949).

MODE OF FORMATION OF 3,4-BENZOPYRENE IN HUMAN ENVIRONMENT

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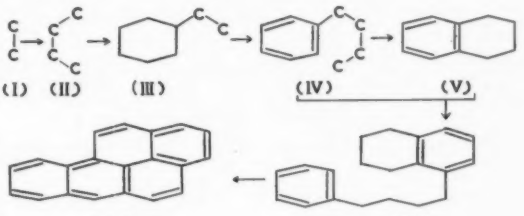
Soot, coal tar, shale oil and some petroleums have long been recognized as the probable cause of certain 'industrial cancers', particularly cancers of the skin. Yamagiwa and Ichikawa[1] demonstrated experimentally that coal tar has cancer-producing activity in laboratory animals, and a pure carcinogenic hydrocarbon, 3,4-benzopyrene (VII), was isolated from coal-tar pitch in 1933[2]. The same hydrocarbon has since been identified in soot[3] and in shale oil[4]. 3,4-Benzopyrene may be chiefly responsible for the cancers caused by soot, coal tar and shale oil; but other carcinogenic hydrocarbons, notably 3,4 : 8,9-dibenzopyrene, 3,4 : 9,10-dibenzopyrene and 3,4-benzofluoranthene have since been identified in coal tar[5].

In recent years 3,4-benzopyrene and other polycyclic aromatic hydrocarbons have been identified in carbon blacks[6], in processed rubber[7], in atmospheric dust[8], in the exhaust gases of petrol and diesel engines[9], in coal gas[10], in coffee soots[11], in human hair wax[12], in oysters and barnacles taken from polluted water[13], in Icelandic smoked food and in the soot from a smoked sausage factory[14], in snuff[15], in tobacco[16] and in cigarette and tobacco smoke[17]. This widespread occurrence of 3,4-benzopyrene in human environment suggests that this hydrocarbon may be implicated in the causation of some cancers in addition to those of industrial origin. The mode of formation of 3,4-benzopyrene therefore seems to be of importance.

The carcinogenic hydrocarbons which occur in human environment seem to be formed only at high temperatures, that is, by reactions under pyrolytic conditions[18]. Several plausible mechanisms for the formation of 3,4-benzopyrene can be postulated, notably the addition of a two-carbon unit to chrysene or to 1,2-benzanthracene, or of a four-carbon unit to pyrene. As a working hypothesis, however, it was suggested[18] that 3,4-benzopyrene may be formed from simple units by a step-wise synthesis, as in (I) → (VII). Possible intermediate compounds would be ethylene or acetylene (for I), butadiene or vinylacetylene (for II), styrene or ethylbenzene (for III), and phenylbutadiene, *n*-butylbenzene or tetralin (for IV and V).



In his classical experiments, Berthelot[19] showed that when acetylene is heated it gives a tar containing benzene and other aromatic hydrocarbons; and Kennaway[20] later showed that tar prepared by passing acetylene and hydrogen through a tube at 700° has powerful cancer-producing properties. It has now been found that tar produced by the pyrolysis of acetylene contains 2 per cent 3,4-benzopyrene[21].

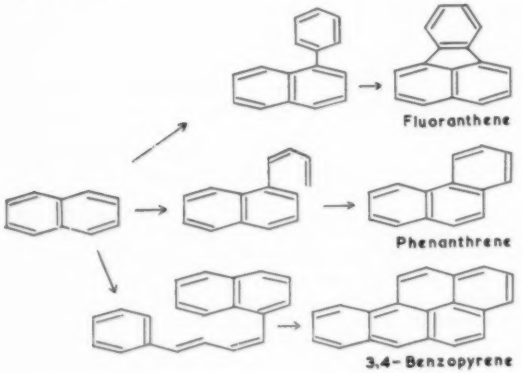
The incomplete combustion of organic compounds often gives some acetylene[22], and acetylene has been detected in tobacco smoke[22]. Nevertheless it seems unnecessary to postulate that the pyrolysis of substances giving benzopyrene invariably proceeds by initial cracking to a two-carbon unit, followed by

re-synthesis. Indeed, it seems more reasonable to suppose that the formation of benzopyrene from dicetyl[24] and from aliphatic tobacco hydrocarbons[25], for example, proceeds by initial cracking to a C_{16} unit, which afterwards gives a C_6–C_4 unit (IV, V), and that two such units then give the key intermediate (VI).

The participation of an intermediate such as (VI) in the formation of benzopyrene has some experimental support. When tetralin is treated with aluminium chloride it gives a complex mixture ('Schroeter tar')[26] which becomes carcinogenic on heating[27], and which has now been shown to contain 3,4-benzopyrene[28]. 6-4′-Phenylbutyltetralin is a major constituent of this mixture; some 5-4′-phenylbutyltetralin (VI) would also be expected, and, although none could be isolated[28], its absence may be due to its too ready conversion into the 3,4-benzopyrene actually isolated. Attempts to synthesize 5-4′-phenylbutyltetralin (VI) have so far been unsuccessful; but the related 1-4′-phenylbutylnaphthalene has been shown to give some 3,4-benzopyrene (albeit in small yield) on pyrolysis[29].

In order to test the hypothesis further, several possible intermediate compounds (butadiene[30], styrene[31], ethylbenzene[30], phenylbutadiene[32], *n*-butylbenzene[30] and tetralin[33]), have been pyrolysed at 700° and the resulting tars analysed. 3,4-Benzopyrene has been identified in each case, but the amount formed varied from compound to compound. Moreover, other compounds (toluene[30], *n*-propylbenzene[30] and indene[34]), which are not direct intermediates in the scheme (I) → (VII), were also found to give some benzopyrene on pyrolysis. However, it may be significant that of the simple alkylbenzenes the one having a C_6–C_4 structure (IV), namely, *n*-butylbenzene, gave the greatest quantity of 3,4-benzopyrene[30].

In each case it has been possible to predict which bonds must have the lowest bond dissociation energies, and will therefore be most likely to break (at the high temperature involved) to yield free radicals. The observed products can then be accounted for by secondary reactions involving these initial or primary radicals. In the pyrolysis of phenylbutadiene[32], for example, phenyl-, butadienyl- and phenylbutadienyl-radicals must be important initial radicals; and naphthalene is an important product of this pyrolysis. The presence of fluoranthene, phenanthrene, and of 3,4-benzopyrene in this tar can then be accounted for by attack of the radicals mentioned on naphthalene, followed by cyclodehydrogenation.



In the same way the alkylbenzenes must undergo primary fission to radicals such as $C_6H_5\cdot$, $C_6H_5CH_2\cdot$, $CH_3CH_2\cdot$, etc., and the products can be accounted for by secondary reactions involving these radicals. Chain re-synthesis must proceed to an appreciable extent, for naphthalene is present in all the tars, and this must be formed from a C_6–C_4 unit. The probable mechanism of chain re-synthesis may be illustrated by the following:

$$Ph\cdot + CH_2 = CH_2 \rightarrow PhCH_2CH_2\cdot$$
$$PhCH_2\cdot + CH_2 = CH_2 \rightarrow PhCH_2CH_2CH_2\cdot$$
$$Ph\cdot + CH_2 = CHCH = CH_2 \rightarrow PhCH_2CH = CHCH_2\cdot$$

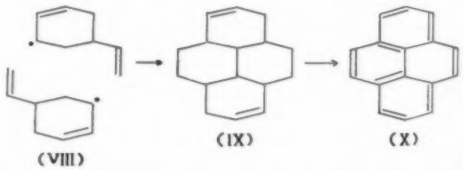
A detailed examination of the results (Table 1) leads to the conclusion that the formation of 3,4-benzopyrene from chrysene or 1,2-benzanthracene by reaction with a C_2 unit can be discounted on the basis of the wide variations in the relative yields of these compounds. The pyrolysis of indene[34], for example, yields little 3,4-benzopyrene, although the yield of chrysene is high and ethylene is present in the exit gases.

Table 1. Yields of 1,2-Benzanthracene, Chrysene, Pyrene and 3,4-Benzopyrene in Pyrolytic Tars

Hydrocarbon pyrolysed	1,2-Benz-anthracene	Chrysene	Pyrene	3,4-Benzo-pyrene
Acetylene	0·4	0·5	6·5	2
Butadiene	0·31	0·39	1·0	0·34
Toluene	0·014	0·03	det.	0·002
Styrene*	—	*c.* 3·3	*c.* 1·9	0·02
Ethylbenzene	0·46	0·55	0·33	0·065
Propylbenzene	0·62	0·96	0·21	0·17
Indene	4·6	31·7	0·10	0·002
Butylbenzene	1·19	1·82	2·0	0·92
Phenylbutadiene	0·89	1·33	—	0·12
Tetralin	2·6	3·43	0·008	0·17

* This analysis was carried out before the development of the technique of chromatography on acetylated paper. The results are therefore not strictly comparable.

On the other hand, it seems that reaction of a C_4 unit (such as butadiene) with a pyrene intermediate may be an important route to 3,4-benzopyrene, in addition to that represented by (I) → (VII). Thus although the pyrene/3,4-benzopyrene ratio is very low for tetralin tar, it is relatively high for most of the other compounds examined. The variation is best explained by assuming that both mechanisms operate to a varying degree depending on the nature of the compound pyrolysed. With the exception of the tetralin tar, in which the benzopyrene may be formed almost exclusively by the route (I) → (VII), it will be noted that tars having high yields of 3,4-benzopyrene also have high yields of pyrene (for example, acetylene, butadiene, *n*-butylbenzene) and tars having low yields of 3,4-benzopyrene have low yields of pyrene (for example, indene).



A suitable pyrene intermediate could be formed as follows. Dimerization of butadiene gives vinylcyclohexene, and two vinylcyclohexene radicals (VIII) could give a hydropyrene (IX). Dehydrogenation of this would give pyrene (X); but addition

of another molecule of butadiene followed by dehydrogenation would give 3,4-benzopyrene.

This work has been supported by the Damon Runyon Memorial Fund for Cancer Research Inc., to whom we express our thanks.

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RADIOLYSIS OF TERPENE HYDROCARBONS: ISOMERIZATION AND POLYMERIZATION OF α- AND β-PINENE

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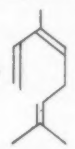
CERTAIN molecular rearrangements characteristic of reactions undergone by mono-terpenoids offer varied examples of processes occurring by carbonium ion mechanisms[1]. In view of recent interest[2–6] in the possibility that reactions induced by ionizing radiation in some organic systems occur in part by ionic species, it seems pertinent to describe some investigations on the radiolysis of α- and β-pinene. All the irradiations have been carried out using cobalt-60 gamma-rays on liquid samples of high purity *in vacuo*.

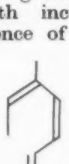
α-*Pinene*,

The products of irradiation at the ambient temperature of the source (about 30° C.) consist of hydrogen, isomers of α-pinene, and a low molecular weight ($\overline{M}_n = 428 \pm 9$) polymer corresponding to the average formation of a trimer. Products of molecular weight intermediate between hydrogen and C_{10} compounds are only formed in minute amounts by comparison with the above, as revealed by gas-liquid chromatographic analysis. Yields of hydrogen and polymer, the latter expressed in terms of monomer disappearance, are 0·56 and 5·0 molecules per 100 eV. respectively; the latter yield can only be considered approximate because of the difficulty of removing the last traces of solvent from the polymer.

Isomerization has been studied in detail by gas-liquid chromatography of the irradiated liquid using poly-propylene sebacate as a stationary phase at 90° C.; in this manner it was possible to analyse for concentrations of isomers lower than 0·1 per cent in the α-pinene. The identification of the individual isomers was accomplished by the collection of appropriate fractions on elution from the column and, after further purification, by examination of the infra-red and ultra-violet spectra of the isolated components. By these procedures,

ocimene  and dipentene

have been identified as the two main isomeric products with initial 100 eV. yields (G values) of 2·2 and 0·9 respectively. The rate of growth of ocimene concentration decreases with increasing irradiation dose, and there is evidence of the formation of

allo-ocimene 

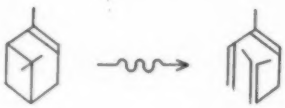
on prolonged irradiation exposure, presumably by further isomerization of the primarily formed ocimene. By contrast, the formation of dipentene is linear with dose up to a concentration of 1·0 per cent in the α-pinene.

Other isomerization or rearrangement products of similar molecular weight are formed in lower yields of which by far the most abundant ($G = 0·6$) is a compound '*X*' with a chromatographic retention time slightly less than that of dipentene, but we have not yet succeeded in identifying its structure; it can be stated, however, that it does not contain terminal group unsaturation (vinyl or vinylidene) and its ultra-violet spectrum does not exhibit evidence of extensive conjugation.

The irradiation of α-pinene at − 196° C. gives *G* values for the formation of ocimene, dipentene and '*X*' equal to 0·8, 1·3 and 0·3 respectively; thus in the solid state the isomerization proceeds more readily to dipentene but is appreciably less facile in favour of ocimene.

It is of related interest that the formation of ocimene, dipentene and allo-ocimene have been observed by Hawkins *et al.*[7] in the vapour phase thermal isomerization of α-pinene using short contact times on an electrically heated element. In the liquid phase it has also been shown[8,9] that the pyrolysis of α-pinene at temperatures exceeding 200° C. leads to the formation of α- and β-pyronenes by the cyclization of allo-ocimene, the latter being itself formed from the initial production of ocimene in the system. By contrast it has been found[10,11] that the homogeneous acid-catalysed isomerization of α-pinene gives mainly monocyclic terpenes, including dipentene, whereas the heterogeneous acid reaction on activated clays gives predominantly camphene; ocimene formation has not been reported under acidic conditions.

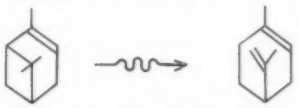
An interpretation of the radiolysis results to allow for ocimene production would be the unimolecular rearrangement of either electronically or vibrationally excited α-pinene molecules by the splitting of the *cyclo*butane ring without the intermediate secession of atomic hydrogen or free radicals; this process can be considered analogous to the mechanism of the thermal decomposition of *cyclo*butane to give two molecules of ethylene[12]. The excited molecules could arise in radiolysis either by direct excitation or by neutralization of the initially created positive ion; it is also feasible that the excited ion undergoes rearrangement to the ocimene structure before neutralization.



(1)

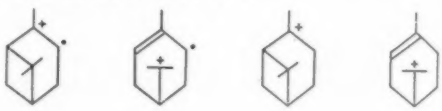
The lower yield of ocimene at − 196° C. relative to room temperature may not be solely due to the increased energy barrier to the isomerization at the lower temperature. Even allowing for the maximum $3n-6$ degrees of vibrational freedom, the energy difference per molecule between − 196° C. and 30° C. would not exceed 1·5 eV., whereas it has been calculated[13] from the Bethe theory that the average energy imparted by fast charged particles to a medium of hydrogen-like atoms is about 5 eV. per direct excitation. A calculation of the activation energy for the first-order thermal decomposition of α-pinene to ocimene and other products from the data given by Hunt and Hawkins[14] gives a value of 37·7 k.cal./mole or 1·6 eV./molecule. It is likely, therefore, that the frequency factor for the rearrangement reaction (1) is adversely affected by the steric confinements of the solid state at low temperature.

The process of dipentene formation is the result of a scission reaction at the gem-dimethyl substituted carbon atom in the *cyclo*butane ring; it can be represented formally as the transfer of a hydrogen atom from one of the methyl groups to the carbon atom at which the *cyclo*butane ring is severed leaving behind an *iso*propenyl group attached at the 4-position in the *cyclo*hexane ring.



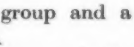
(2)

Before proceeding with a more detailed consideration of reaction (2) it is necessary to digress on the nature of the positive ion likely to be formed on irradiation of α-pinene. Chang, Yang and Wagner[5] have suggested that the molecular ion primarily formed on radiolysis of olefin systems is the radical ion produced by localization of the positive charge at the unsaturated carbon atoms; thus for α-pinene it would be represented by I.



I II III IV

On the basis of the well-understood preferential rearrangement[1] of the carbonium ion III (as produced by proton addition to α-pinene) to the form shown in IV, it can be argued that I would rapidly rearrange to the analogous form shown in II. The mass spectral pattern for α-pinene (A.P.I. Research Project 44, Serial No. 465) reveals that less than 5 per cent of the parent ion is detected, and the most abundant ion is of mass 93, which corresponds to the removal of the

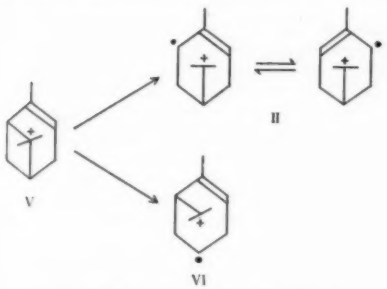


group and a hydrogen atom from the parent. However, in the rarefied conditions of a mass spectrometer chamber and because of the relatively large time-interval before detection (10^{-5} sec.), the initial ion is much more prone to extensive decomposition by endothermic step-wise fragmentation than in the liquid phase; in the latter case it is suggested that the more relaxed conditions prevent the further decomposition of the rearranged ion II prior to the eventual neutralization process which has been estimated for some liquid systems to occur in 10^{-12}–10^{-13} sec. (ref. 15). A similar conclusion can be reached as to the likely nature of the positive ion species by comparison with mass spectral findings for alkanes containing quaternary substituted carbon atoms. As an example, the parent ion for neo-pentane is almost non-existent (about 0·01 per cent) in the mass pattern and the main peak is due to the tert-butyl carbonium ion, $C_4H_9^+$. In explanation, Field and Franklin[16] have calculated that the decomposition of the parent ion according to (3) is almost thermoneutral whereas an alternative mode of dissociation given by (4) is 72 k.cal./mole endothermic.

$$neo - C_5H_{12}^+ \rightarrow t - C_4H_9^+ + CH_3^\bullet \quad (3)$$

$$neo - C_5H_{12}^+ \rightarrow t \cdots C_4H_9^\bullet + CH_3^+ \quad (4)$$

An ion dissociation process of type (3) involving negligible activation energy is expected to proceed within a period determined only by the frequency factor of the reaction; for a simple bond dissociation in an ion, Stevenson[17] has indicated that the frequency factor is 10^{14} sec.$^{-1}$. Accordingly, even in the liquid state, reactions of type (3) may be expected to occur before neutralization, whereas endothermic processes are largely precluded by the short lifetime of the ion (about 10^{-13} sec.). A fuller discussion of the implications of fast ion dissociation reactions for the radiation chemistry of branched hydrocarbon systems, including polymers, will be given by one of us in a future publication[18]. The relevance of a reaction analogous to (3) for α-pinene can be seen by considering the mode of decomposition of the parent ion V; in this instance it is possible to form two alternative carbonium ions by the scission of the *cyclo*butane ring on either side of the gem-dimethyl group, constituting the quaternary substituted carbon atom in the 'centre' of the molecule, as represented in (5) and (6).

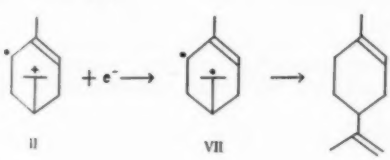


(5)

(6)

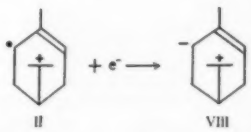
The removal of a methyl group in the above scission is discounted since the largest attached group is generally the most labile in the breakdown of a branched parent ion[16]. Concerning the alternative processes (5) and (6), the former will clearly be favoured by the greater resonance stability of the allylic radical as represented by the equivalent mesomeric structures shown for II. It can therefore be concluded that the most likely ion to be produced in the radiolysis of α-pinene can be represented by II whether the positive charge is initially considered to occur at the double bond or at the tetra-substituted carbon atom; since these two locations represent regions of high electron density in the original molecule it follows that the positive charge would tend to concentrate preferentially at these locations.

The neutralization of the ion-radical II by the conjugate electron can be represented in three ways: first, by neutralization of the carbonium ion to give a biradical VII followed by disproportionation involving hydrogen atom transfer from one of the methyl groups to the ring position and the concomitant formation of an *iso*propenyl group as represented in (7).



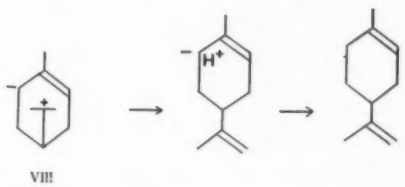
(7)

A criticism of (7) is that the ease of hydrogen atom transfer from the methyl group to the ring position may not be sterically favoured. Secondly, electron capture may proceed at the radical location in II to create an ion-pair VIII in the same molecule, as shown in reaction (8):



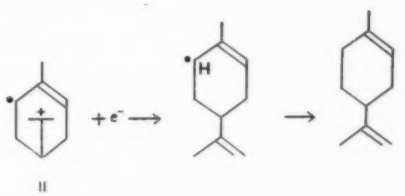
(8)

the postulated relative stability of VIII as an intermediate state is assumed to be derived from the delocalization of the positive charge on the carbonium ion in this instance, accruing from the inductive effect and hyperconjugation of the attached groups. The contribution of solvation forces to the increased stability of tri-substituted carbonium ions may also be of significance[19] although the low dielectric constant of α-pinene is not particularly favourable in this case. Apart from the obvious possibility of ionic combination to re-form α-pinene, there exists the likelihood of proton expulsion from the carbonium ion and concomitant capture of the proton by the negative ion held in close juxtaposition by the coulombic forces of the ion-pair; these latter processes bear an obvious parallel to the classical S_N1 and $E1$ mechanisms originally invoked by Hughes and Ingold[19] to explain the reactivity of tertiary-butyl halides. So, by analogy, the process of dipentene formation as in (9) can be regarded as equivalent to the elimination reaction found to accompany substitution processes in carbonium ion reactions[20].



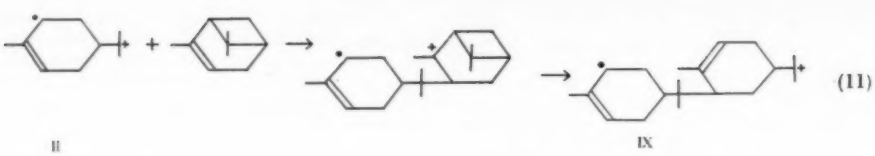
(9)

The third mechanism of neutralization of II is essentially a variant of the previous one in which the electron functions as a nucleophilic entity and attracts a proton from the carbonium ion, thus forming a hydrogen atom. Subsequent combination of the hydrogen atom and the radical would again lead to dipentene as in (10).



(10)

If prior to neutralization the carbonium ion II, or the ion-pair VIII, could transfer its proton to another molecule of α-pinene, then there would be the possibility of the isomerization of several α-pinene molecules per initial ion according to the usual acid-



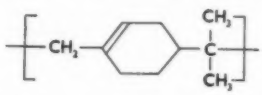
II IX (11)

catalysed mechanism[1] of dipentene formation. However, consideration of the yields obtained suggests that the frequency of transfer is small compared with the initial number of ions formed; in this connexion the slight increase in yield at − 196° C. over room temperature conditions may be an indication of some migration in the solid state arising from a longer life-time of II before neutralization. The apparent absence of an isomer of dipentene in the products as would be formed by the alternative mode of scission of the *cyclo*butane ring in reaction (6) is in agreement with the mechanism proposed above.

The negligible yields of methane, ethane and other fragmentation products lead us to argue against extensive radical reactions in this system following from the creation of methyl radicals in a primary act. Polymer formation can be formulated as a consecutive ion-molecule reaction (11) in which the initiating species is the carbonium ion II and the termination process occurs, on average, after the addition of two molecules of α-pinene; the subsequent neutralization of the propagating carbonium ion IX could, by analogy with the isomerization process, give rise to either a diradical, an ion-pair or a radical plus hydrogen atom. If a diradical is formed the molecule could be stabilized by olefin group formation as proposed by Chang, Yang and Wagner[5]. The possibility of proton transfer from a propagating species IX, which thereby terminates, to another α-pinene molecule, thus inducing either a new polymerization chain or isomerization to dipentene, is precluded by the magnitude of the measured yields.

β-*Pinene*.

On irradiation, β-pinene polymerizes readily at ambient room temperature with a large yield, G_{initial} (-monomer) about 2,000, to very high conversions (90 per cent)[21]. If oxygen or water is present the polymerization is inhibited. The polymer was isolated in two fractions according to whether it was insoluble or soluble in the remaining monomer. In a typical experiment, the yield of precipitated polymer was slightly greater than that of the soluble fraction. The insoluble polymer consists of a white solid showing birefringence to polarized light and having a melting point exceeding 200° C., at which temperature it does not undergo obvious decomposition in the absence of oxygen; it is insoluble in most organic solvents of boiling point less than 100° C. Analysis of the carbon and hydrogen content on samples which had either been melted *in vacuo* after irradiation (ostensibly to remove any free radicals formed by irradiation of the precipitated polymer during the polymerization) or been kept in the absence of air gave exact concordance with the formula $(C_{10}H_{16})_x$; however, the analysis of samples further exposed to atmospheric oxygen for more than a week indicated that oxidation had occurred and this was confirmed by infra-red evidence of the formation of $\diagdown C = O$ groups on standing. Infra-red examination of the precipitated polymer in a potassium bromide disk immediately after irradiation revealed the spectrum to contain all the absorption bands listed[22] for a sample of β-pinene polymer obtained either by Friedel–Crafts or Ziegler type polymerization, supporting the structural assignment

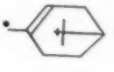


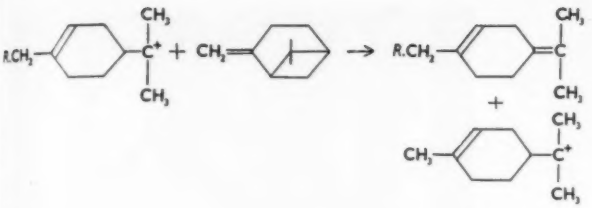
as the most probable repeating unit in the polymer structure. Other absorption bands not characteristic of the Friedel–Crafts polymer and also absent in a sample of commercially available β-pinene resin are found to be consistently present in all polymers formed by irradiation. The soluble polymer was found to have a softening point in the range 80–105° C. and the infra-red spectrum was similar to that of the precipitated fraction; molecular weight measurements by an ebullioscopic method in chloroform as a solvent gave a value of 550. For the total polymer, the molecular weight determinations are uncertain owing to its relative insolubility but a value of 1,450 can be inferred from the data for the portion of sample dissolving in chloroform at its boiling point.

Detailed information as to the polymerization kinetics is not as yet complete; but the results obtained hitherto using pure monomer, prepared by fractional distillation and passage down a silica gel column, suggest that the polymerization is reproducible and not largely affected in terms of yield per total dose either by dose-rate or cell diameter. Irradiation at a mean temperature of −40° C. resulted in a two-fold reduction of polymer yield by comparison with the room temperature results.

By comparison with the ease of the polymerization reaction, the isomerization induced by irradiation of β-pinene is of negligible occurrence and only very low yields ($G < 0{\cdot}1$) of dipentene and other isomers have been observed.

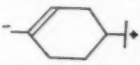
β-Pinene is recognized[23] as typical of a group of monomers that undergo polymerization by ionic initiators such as Friedel-Crafts catalysts but not by free radical agents; for example, the addition of carbon tetrachloride to β-pinene[24] initiated by benzoyl peroxide leads to an almost quantitative yield of 1 : 1 addition product without the formation of polymer, in contrast to the partial polymerization (telomerization) of vinyl monomers in similar circumstances[25].

By complete analogy with the previous discussion for α-pinene, the rearranged positive ion for β-pinene is given by $X =$  ; it is postulated that



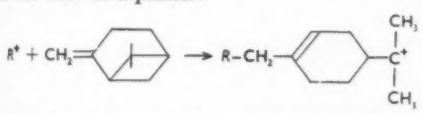
(12)

either this carbonium ion-radical *X* or the ion-pair

XI =  constitutes the original

initiating species for polymerization. The results of this investigation show unequivocally that the high yield of relatively low molecular weight polymer fraction corresponds to the occurrence of a large number (about 100) of separate polymerization chains per 100 eV., and since the number of primarily formed initiators cannot attain such high values the obvious implication is that the polymerization is sustained by transfer of an initiating species from a propagating polymer chain to a monomer. Such transfer mechanisms are well known to occur in ionic polymerizations[26] by proton transfer and are illustrated in this instance by reaction (12).

The propagation step can be written for the general case of the ion R^+ as in (13) and involves the opening of the *cyclo*butane ring as previously conceived for the case of α-pinene.



(13)

For high polymerization yields to be effected by a cationic mechanism in radiation chemistry it is necessary either that the polymerization chain, including possible transfer, occurs in a time of about 10^{-12} sec., that is, before neutralization, or else the conjugate electron must be stabilized for sufficient time to allow the polymerization to proceed in a purely thermal manner before termination. Anderson[4] has suggested the former explanation for the polymerization of butadiene, whereas Dainton[6] and also Davison, Pinner and Worrall[3] have put forward the view that the electron is probably trapped by sub-microscopic solid particles in liquid *iso*butene. Another general possibility arising from this present work is that of the creation of sufficiently stabilized ion-pair initiators.

According to the mechanism depicted here, the crucial factor governing the magnitude of isomerization and polymerization in these monomers on radiolysis is the ease of transfer of a proton from a carbonium ion to the monomer.

We wish to thank Dr. W. Wild for his interest during the course of this work.

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SILKWORM RAISING ON ARTIFICIAL FOOD

By TOSHIFUMI FUKUDA, MITSUMASA SUTO and YOSHIKICHI HIGUCHI

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ATTEMPTS to rear silkworms, *Bombyx mori*, on an artificial food have been made for a long time. However, there are no reports that silkworm larvæ have been reared on artificial food only from hatching to spinning. Recently, Hamamura[1] and Ito[2] found that silkworms will eat agar-agar containing nutriments for two or three days, and pointed out that this phenomenon is of value for study of food selection of the silkworm larva. Yoshida *et al.*[3] have succeeded in rearing silkworms on artificial food, from hatching to the fourth day of the fifth instar (maximum). They could not, however, get the silkworms to produce cocoons. So we reared silkworms by improving the composition of the artificial food and the method of the silkworm raising devised by Yoshida *et al.*, and succeeded in making silkworm larvæ produce 36 cocoons, starting from 110 worms hatched from eggs. The silkworms pupated, and the fertilized moths laid their eggs.

The composition of the basal artificial diet is given in Table 1.

To this basal diet (8·5 gm.) was added 5 ml. of 20 per cent sucrose solution, and 10 ml. of the inhibitor solution. The inhibitor solution was prepared by

Table 1. Composition of the Basal Diet

Powdered mulberry leaves*	5 gm.
Potato starch†	1·5 gm.
'Kinako' (a powder of parched soy bean)†	1 gm.
'Koridofu' non-fat (frozen soy bean-curds)‡	1 gm.

* Leaves picked from mulberry trees ('Kokuso' No. 19 and 'Ichinose') were dried at not more than 40° C. under vacuum, and then powdered to pass through an 80-mesh sieve.

† Potato starch and 'Kinako' respectively were passed through an 80-mesh sieve.

‡ The dried 'Koridofu' was treated with ether to remove fat, and then powdered to pass through an 80-mesh sieve.

dissolving 0·1 gm. of vitamin K_3, 0·2 gm. of sodium dehydroacetate and 0·2 gm. of sodium sorbate in 100 ml. of distilled water by heat. This inhibitor is necessary to stabilize the artificial diet, and to prevent its decomposition. The mixture was stirred well with a glass rod. The artificial food prepared was sterilized in an autoclave for 15 min., and stored in a refrigerator. The artificial food thus prepared remains good for a week.

A piece of wet filter paper was placed in the bottom of a Petri dish ready for raising the silkworms. This was covered with a piece of paraffin paper. The artificial food was placed on the paraffin paper, then the newly hatched worms (the hybrids between *Si* 115 × *Si* 124 and *Nichi* 122 × *Nichi* 124) were placed on it and reared there. The dish, covered with a lid, was placed in a sterilized box at a temperature of 25° C. and at 92–96 per cent humidity. The artificial food was cut in slices 0·1 mm.–0·3 mm. thick. Each slice prepared was sterilized in an autoclave for 15 min., and then stored in a refrigerator for 12 hr. before use. Slices were fed to the silkworms twice daily at 12-hr. intervals.

The time between hatching of silkworm larva and spinning a cocoon was 45 days. This is about twice as long as the same race reared on mulberry leaves. The pupal period was 14 days. Fig. 1 shows the silkworms at the eighth day of the fifth instar, three days before spinning, eating the artificial food. Among 110 newly hatched silkworms, 36 spun their cocoons, and 24 silkworms underwent the pupa stage (♀2 and ♂22) and 24 became moths (♀2 and ♂22). The weights of the matured larva, the pupa (at the sixth day after pupation), and the moth were respectively 1·13 gm., 0·62 gm. (♂), and 0·33 gm. (♂). Fig. 2 shows the matured larva, the pupa, the moth, the cocoon, and the eggs obtained by using artificial food. The weight of the cocoon fibres produced by one silkworm was 86 mgm. (130 mgm. maximum), and its value is about 20 per cent (about 40 per cent) of that in the case of the silkworm reared on mulberry leaves. The length of a silk filament unwound from one cocoon was about 450 m. In a matured larva, the pair of silk-glands developed quite well. The number of the eggs laid by one moth was 227. One egg was 1·291 mm. long by 0·952 mm. wide.

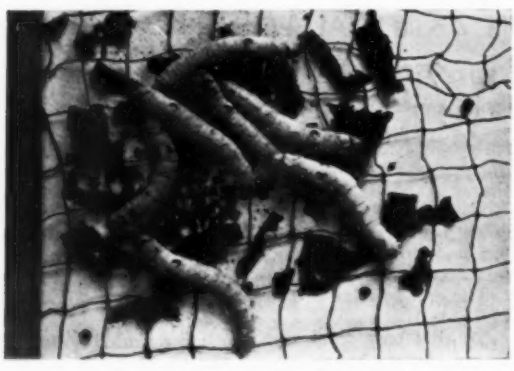

Fig. 1. Silkworms eating artificial food

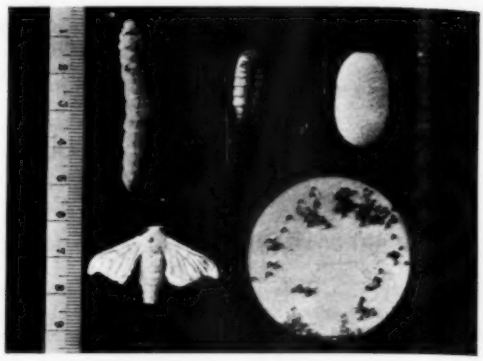

Fig. 2. Mature larva, pupa, moth, cocoon and eggs raised on artificial food

We succeeded in rearing silkworms, from hatching to the moth, on artificial food; but this fact does not mean that the artificial food used is the most suitable one for silkworm raising. The weight of the cocoon fibres obtained at present is not very much. By improving the composition of the artificial food used, however, it may be possible for the silkworm to produce a much larger amount of cocoon fibres and eggs. Concerning the artificial food, especially the powdered mulberry leaves, it is interesting to note that the nutritive value of the powdered leaves decreases remarkably when the powder has been at room temperature for a long time, stored in a glass bottle. In the case of silkworms at the first age reared on an artificial food containing this powder, a great part of the silkworms would not have been raised to the second age. This phenomenon seems to be of interest in relation to the food selection of the silkworm larva. In the artificial food used the powdered mulberry leaves is present in relative abundance (about 20 per cent). It seems to be desirable, if possible, to replace the mulberry leaf powder with some other simple nutriment.

We also tried to rear the wild silkworm (*Attacus ricini*) with artificial food, and succeeded in rearing it until just before the fifth instar, starting from the hatched silkworms. The artificial food used for the wild silkworms was one in which the powdered mulberry leaves were replaced by powdered castor-oil (*Ricinus communis*) leaves, which is used in artificial food for *Bombyx mori*.

We wish to thank Dr. Eikichi Hiratsuka, Dr. Tadao Yokoyama, Dr. Tokutaro Yoshida, Mr. Michio Matsuoka (Sericultural Experiment Station, Tokyo), Dr. Shojiro Ishii (National Institute of the Agricultural Science, Tokyo), Prof. Marcel Florkin (University of Liège, Belgium) and Prof. Jean Leclercq (University of Liège) for their kindness and advice. Our thanks are especially due to Mr. Koichi Kimura for helping us with the preparation of artificial food.

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ENZYMATIC ACTIVATION OF A HORMONE-LIKE RESPONSE IN *HYDRA* BY PROTEASES

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THE mechanism by which excitatory compounds act on cells is not clearly understood. (In view of recent controversy[1], we use the general term 'excitatory compound' as a strictly operational expression to signify any specific substance which functions naturally in eliciting a co-ordinated biological response; thus hormones are the most studied group of excitatory compounds.) Some recent work, which has been concerned with the action of exogenous excitatory compounds on tissue slices[2], is in agreement with the belief that cellular integrity is essential for excitatory compounds to elicit their response[3]. As another approach to this problem, we have been studying the action of an exogenous excitatory compound on an intact, simple, aquatic animal which has not developed past the tissue-level of organization[4]. This system involves the action of reduced glutathione in eliciting the feeding reflex in *Hydra*. In this article we present results which demonstrate that certain proteases can simulate enzymatically the action of reduced glutathione on the live animals.

Small amounts of reduced glutathione have been shown to act as a 'feeding hormone' in eliciting the feeding reflex in *Hydra*[5] and in other hydrozoans[6]. As pointed out by Loomis[5], the feeding reflex involves the well-co-ordinated muscle movements necessary for the ingestion of prey by the *Hydra*. In Nature, the *Hydra* can carry out the feeding reflex only by puncturing the prey with spear-like nematocysts (stinging organoids). After the prey, such as a *Daphnia*, is punctured by the discharged nematocysts, fluids ooze from the wound into the water surrounding the *Hydra*. Reduced glutathione, which is present among these fluids, induces the *Hydra* to carry out the feeding reflex resulting in the ingestion of the wounded prey. Since reduced glutathione is present only in live or recently killed food, the carnivorous *Hydra*, which lacks the specialized sensory organs found in higher forms, is enabled to select living food for ingestion through the stimulation produced by reduced glutathione. Loomis[5] has demonstrated that the experimental addition of reduced glutathione to *Hydra* will induce the animal to ingest even dead food which is devoid of endogenous reduced glutathione.

In the experiments to be described, the feeding reflex is measured quantitatively by averaging the lengths of time (in minutes) during which the mouths of all the *Hydra* tested remained open in response to the protease. This is the procedure by which the normal response to reduced glutathione is measured[4]. Male *Hydra littoralis*, reproducing asexually in laboratory mass cultures[7], were used in all the experiments. The assay consisted of the following procedure: five *Hydra*, which had been starved for one day, were rinsed three times in 30-ml. aliquots of standard culture solution (see below) during a 20-sec. period; then, in one drop of culture solution, they were placed in 2 ml. of the solution to be tested ('test solution'), and were observed immediately through a binocular dissecting microscope. The lengths of time that the mouths of the *Hydra* remained open were recorded, and the average was taken. The test solution was contained in the spherical cavity (38 mm. diameter) of a 3 in. × 2 in. depression slide. The microscope was set at × 19·5 magnification.

The enzymes and proteins used were: papain (twice crystallized), trypsin (twice crystallized), pan-protease preparation, aminotripeptidase, crystalline soybean trypsin inhibitor, peroxidase, collagenase and hyaluronidase (Worthington Biochemical Corp., Freehold, N.J.); chymotrypsin, ficin, pepsin (twice crystallized), cathepsin C, carboxypeptidase, carbonic anhydrase, catalase, α-amylase, and egg albumin (Nutritional Biochemicals Corp., Cleveland, Ohio); urease (twice crystallized), casein and hæmoglobin (Mann Research Laboratories, N.Y.); cellulase, alcohol dehydrogenase (twice crystallized), lactic dehydrogenase, hexokinase and bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.); elastase (Warner-Chilcott, N.Y.); muscle aldolase and α-glycerophosphate dehydrogenase (Southeastern Biochemicals, Inc., Augusta, Ga.), and gelatin (Eastman Chemicals, Rochester, N.Y.). The enzymes and proteins were used at a concentration of 0·1 mgm./ml. unless stated otherwise. All the enzymes were dissolved in the standard culture solution of 10^{-3} *M* calcium chloride and 10^{-4} *M* sodium bicarbonate at *p*H 7·3–7·8, and were dialysed overnight at 4° C. against four litres of this solution. The dialysis was carried out in order to remove any possible contaminating reduced glutathione.

The experiments are listed in Table 1. Experiment 1*a* is a control and shows that in the presence of 10^{-5} *M* reduced glutathione the *Hydra* kept their mouths open for about 21 min.; this was the normal feeding reflex. Results obtained using crystalline papain are given in Experiment 2. The dialysed enzyme (20 μgm./ml.) elicited only a negligible response (Exp. 2*a*). Since reducing agents are known to activate the proteolytic activity of papain[8], the enzyme was incubated with 10^{-3} *M* cysteine for 5 min. A feeding reflex of 20 min. duration resulted (Exp. 2*b*). Cysteine alone, as previously demonstrated by Loomis[5], also was shown here to be without effect (Exp. 1*b*). No response was elicited if the enzyme and cysteine mixture were heated for 5 min. at 100° C. (Exp. 2*c*). (It should be emphasized that the sulphydryl group has recently been shown to be unnecessary for the activity of reduced glutathione on the *Hydra*. These analogues, ophthalmic acid and norophthalmic

acid, which have methyl and hydrogen respectively replacing the sulphydryl group of reduced glutathione, can induce the feeding reflex[9]. On the other hand, reduced asparthione, which contains one less methylene group than reduced glutathione, is inactive[5]. Thus, it appears that the receptor is very specific for the molecular size and shape of reduced glutathione, and does not depend upon the sulphydryl moiety.) Ficin, another proteolytic enzyme activated by cysteine[10], also behaved similarly to papain (Exp. 3). The dialysed ficin did not elicit the response (Exp. 3*a*); addition of cysteine to the enzyme resulted in a significant response (Exp. 3*b*); and boiling destroyed the ability of ficin to elicit the feeding reflex (Exp. 3*c*).

Results obtained using crystalline trypsin are given in Exp. 4. Dialysed trypsin elicited a 17·8-min. response (Exp. 4*a*). The reaction was carried out at *p*H 5·7, which was found to be more favourable for the action of trypsin on the *Hydra*. The action of trypsin in eliciting the feeding reflex was inhibited by previously incubating the enzyme with crystalline soybean trypsin inhibitor (Exp. 4*b*). This action of the trypsin inhibitor was due to the inhibition of trypsin rather than to any deleterious action of the inhibitor on the *Hydra*; for trypsin inhibitor had no effect on the normal response stimulated by reduced glutathione. The activity of trypsin was reduced only slightly by heating at *p*H 5·7 for 5 min. at 100° C. This is in agreement with previous work concerning the relative heat stability of crystalline trypsin at an acid *p*H (ref. 11). However, boiling the enzyme solution at *p*H 7·5 for 5 min., and then adjusting the *p*H to 5·7, reduced the enzymatic activity, and only an 8-min. response occurred.

In summary, the results listed in Table 1 present evidence that the stimulation of the feeding reflex by papain, ficin, or trypsin followed closely the characteristics of the individual enzymes. Papain and ficin acted only in the presence of a reducing agent. Trypsin activity was inhibited by trypsin inhibitor, and was not inhibited by boiling this heat-stable crystalline enzyme at an acid *p*H. It is concluded that the feeding reflex was activated by the proteolytic activities of these enzymes.

In addition to papain, ficin and trypsin, other proteases elicited the feeding reflex in *Hydra*; the pan-protease preparation (*p*H 5·7), 24·5 min.; chymotrypsin, 8 min.; elastase, 1·4 min. Pepsin and cysteine-activated cathepsin *C* were without effect, as were the peptidases carboxypeptidase and aminotripeptidase. Egg albumin was observed on one occasion to elicit a questionable negligible response. None of the 18 other proteins mentioned could elicit a response. The results of this partial survey of proteins suggests that, under the conditions described in this communication, the activation of the feeding reflex is due to the enzymatic action of proteases, and not to any non-specific effect of proteins in general.

The results presented in Table 1 may give the erroneous impression that the opening of the mouth which occurred in the presence of proteases was an abnormal response to a toxic compound. For example, some deleterious compounds have been observed to cause abnormal opening of the mouth, but they did not cause the characteristic feeding response. These poisoned 'gaping' *Hydra* could not carry out the highly co-ordinated movements necessary to ingest dead food (Lenhoff, H. M., unpublished work). In contrast, the *Hydra* reported in the positive

Table 1. Activation of the Feeding Reflex by Proteases

The experiments were performed as described in the text. The reactions were carried out in the standard culture solution. All solutions were maintained at a near neutral *p*H except for those used in Exp. 4; the latter solutions were adjusted to *p*H 5·7

Experiment	Test solution	Average time mouth of *Hydra* open (min.)
1*a*	10^{-5} *M* reduced glutathione	20·6
b	10^{-3} *M* cysteine	0
2*a*	20 μgm./ml. papain	0·1
b	20 μgm./ml. papain + 10^{-3} *M* cysteine	19·8
c	20 μgm./ml. papain + 10^{-3} *M* cysteine, boiled 5 min. at 100° C.	0
3*a*	0·1 mgm./ml. ficin	0
b	0·1 mgm./ml. ficin + 10^{-3} *M* cysteine	12·3
c	0·1 mgm./ml. ficin + 10^{-3} *M* cysteine, boiled 5 min. at 100° C.	0·1
4*a*	0·1 mgm./ml. trypsin	17·8
b	0·1 mgm./ml. trypsin + 0·1 mgm./ml. trypsin inhibitor	0

enzyme experiments listed in Table 1 (Exp. 2*b*, 3*b* and 4*a*) were able to manipulate and ingest dead food which is devoid of any endogenous reduced glutathione. The ability to ingest food is the most important characteristic of the feeding reflex as determined by Loomis[5]. It should also be noted that the deleterious compounds which cause this gaping usually killed the *Hydra* within a few hours (Lenhoff, H. M., unpublished work). In contrast, the *Hydra* which had been exposed to the proteases for more than 24 hr. in the experiments reported here remained intact and alive.

Preliminary experiments have demonstrated that either trypsin or papain can stimulate isolated *Physalia* gastrozooids to carry out their characteristic feeding reflex (Lenhoff, H. M., unpublished work). Recently these structures have been shown to respond to reduced glutathione in a manner similar to *Hydra*[6].

It seems strange that a response which is so complex and highly co-ordinated as the feeding reflex can be set off by two different classes of substances: the normal excitatory compound—reduced glutathione, and proteolytic enzymes. A consideration of possible mechanisms by which proteases could activate the feeding reflex may give insight into the normal mechanism by which reduced glutathione acts. One possibility may be that the enzymatic cleavage of certain peptide bonds alters the permeability of the cell membrane and triggers the response. Another explanation is that key enzymes, which are needed to initiate the feeding reflex, may be released from their naturally occurring protein inhibitors. For example, proteases are known to activate deoxyribonuclease[12], fructose-1,6-diphosphatase[13], enzymes involved in blood clotting[14] and precursors of proteolytic enzymes[15]. Other enzymes have been shown to exist in combination with heat-labile protein inhibitors[16]. Lastly, co-factors could be released in the same fashion. In the present case, reduced glutathione released from the bound state could be such a co-factor.

As regards the site of action of the proteases, it should be borne in mind that the feeding reflex evoked by the proteases involves many interrelated processes. Most probably these processes could not be initiated by the action of the proteases on any one of the terminal proteins involved in the co-ordinated response. Therefore, it appears that the protease

acts on some primary protein which sets off the chain of reactions involved in the complex feeding mechanism. A likely candidate is the reduced glutathione-receptor, or a protein closely associated with the receptor.

Finally, it does not seem inconceivable that the proteolytic activation of a response normally induced by an excitatory compound, such as occurs with *Hydra*, may be found to occur in other systems. The results presented in this communication make it tempting to speculate that a basic underlying mechanism by which some excitatory compounds act on cells involves a proteolytic step.

Note added in proof. After this article was submitted, other examples of systems in which proteases stimulate specific responses came to our attention. Trypsin has been shown to activate wound repair activities of corneal stroma cells; the enzyme was postulated "to lead to the release of some factor(s)" which stimulate these activities[17]. More recently, ficin has been reported to stimulate *in vitro* corticosteroid production by rat adrenal tissue[18]; *in vivo*, adrenocorticotrophic hormone carries out this function.

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STRUCTURE AND SYSTEMATIC POSITION OF THE AUSTRALIAN BROWN ALGA, *NOTHEIA ANOMALA*

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NOTHEIA anomala Harvey and Bailey[1], 1851, was first collected by the United States Exploring Expedition at the Bay of Islands, New Zealand. It grows exclusively on *Hormosira banksii* f. *sieberi* at a low littoral level and in low rock pools on rough coasts, along southern Australia and in New Zealand. Previous studies of *Notheia* have been made by Barton[2], Mitchell[3], Gruber[4] and Williams[5].

The specific name is most applicable to this alga, which has previously always been classed in the Fucales, usually in a family with *Hormosira*. *Notheia* shows a group of three apical cells (which protrude at the branch apex and are not sunk in a depression as in the Fucales), conceptacles scattered over the thallus, and the supposed formation of oogonia with eight eggs, and according to Barton[2], of antheridia with numerous sperms. The branches, which issue from the conceptacles, are a further anomalous feature.

The discovery, while examining living material, that the 'eggs' are motile zooids, made a thorough investigation of *Notheia* necessary. This account gives preliminary results, which, while not complete, clarify many features and show that *Notheia* is not a member of the Fucales but is a distinctive type of the Heterogeneratae. The structure and reproduction will be discussed briefly here and will be described more fully elsewhere.

Each of three protruding apical cells segments latero-posteriorly, and the tissues derived from each apical cell can be recognized in both transverse and longitudinal sections for some distance from the apex, due to extensive mucilage development between the three sectors. Some 15–20 cells from the apex this distinction is lost. Derivatives of the apical cells segment periclinally to form rows of cells which develop three tissues—medulla, cortex and peripheral layer which remains meristematic. The development of the cells is largely filamentous and not parenchymatous; but in older medullary tissue divisions in different planes may occur giving a truly parenchymatous tissue. Increase in thickness of the thallus is largely due to the meristematic peripheral layer, the cells of which divide first by a longitudinal-oblique wall (not reaching the base of the cell), followed by a periclinal division near the base of the larger cell. This results in dichotomous branching, the cells remaining connected by large pit connexions, similar to those of the Florideae. This dichotomous branching is readily visible throughout the cortex in hand sections, but thin microtome sections obscure it.

Branching occurs largely but not entirely from within the conceptacles, resulting in a profusely and irregularly branched plant up to 12 cm. high. The plants originate from within or near the ostioles of conceptacles of *Hormosira* plants. The branches are constricted within and immediately outside the conceptacles, but broaden considerably 1–2 mm. outside; here the cortex is fully developed. Occasionally, however, true branches, up to three per plant, occur; here the thallus divides into two with no decrease in thickness or association with a conceptacle. The common branches may be referred to as 'conceptacular branches' and reasons will be advanced later for suggesting that these are new plants and not real branches.

Conceptacles occur profusely scattered over the thallus. They originate by enlargement and trans-

verse division of a peripheral cell; the upper cell divides to form a hair and the lower divides longitudinally and is overgrown by surrounding cells to form the conceptacle. Most of the lining cells of the conceptacle produce paraphyses which protrude through the ostiole as a dense tuft.

Sessile reproductive organs occur on the wall of the conceptacles. Most common are those containing 8 zooids, while slightly smaller ones with 64 zooids vary from plentiful to absent in some conceptacles. The larger organs measure 48–78μ by 12–30μ and are not oogonia but are macrozoosporangia, liberating 8 pyriform macrozoospores through basal, or occasionally lateral or terminal, pores. The macrozoospores are first extruded in a gelatinous envelope, and when they escape are sluggishly motile by means of a long anterior flagellum and somewhat shorter posterior one, the attachment being lateral but close to the pointed apex. Macrozoospores contain 2–4 chromatophores, a single eye-spot and measure 12–27μ × 5–12μ.

The 64 zooids, formed in organs slightly smaller than the macrozoosporangia, are actively motile, almost colourless (with one pale chromatophore), with an eye-spot and two unequal flagella attached near the apex. They measure 6–10μ by 4–6μ. These bodies settle after one-half to several hours but have never been observed to develop further and their nature is still unknown.

During repeated observations no fusion between the large and small zooids has been observed, although they were moving close together.

The macrozoospores germinate in culture into branched, filamentous gametophytes, with erect colourless hairs. The cells of the prostrate parts measure about 12μ in thickness, are 2–4 times as long as broad and contain several brown chromatophores. Uniseriate, erect reproductive bodies are produced on older filaments, each cell producing a single zooid which is liberated apically through the series of fertile cells. These zooids are pyriform, about 8μ × 6μ and biflagellate with lateral, unequal flagella, similar to the macrozoospores. These gametophytes and reproductive cells are very similar to the gametophytes and uniseriate gametangia of *Spermatochnus paradoxus*[6].

Branched, filamentous growths such as those found in culture occur frequently within the conceptacles of *Notheia*, as was observed by Barton[2]. These filamentous growths are usually entirely within the conceptacle, ramifying among the paraphyses and lining cells, but with their colourless hairs sometimes protruding from the ostiole. Occasional empty uniseriate reproductive cells have been seen on them.

Satisfactory chromosome counts have not yet been obtained, but some 26–30 chromosomes have been observed in vegetative cells, about 20–24 in paraphyses and 12–15 in later divisions in the macrozoosporangium. The thallus of *Notheia* is probably diploid, and the macrozoospores and macrogametophyte haploid.

Until the nature of the microzooids and the zooids from the uniseriate gametophytic reproductive organs is known the full life-cycle of *Notheia* will remain uncertain. It seems likely that the macrogametophytes bear uniseriate gametangia producing a single gamete per cell. The behaviour of the microzooids towards these gametes has not yet been observed.

From the known facts, it appears likely that the macrozoospores germinate into macrogametophytes largely within the conceptacles, and possibly fertilization also occurs within the conceptacles, giving rise to a zygote which grows from the wall of the conceptacle. If macrogametophytes develop outside the conceptacles and fertilization occurs externally, the zygote probably is unable to develop without being associated with *Notheia* or *Hormosira*.

This speculation agrees with the development of new conceptacular branches. Commonly one, sometimes two or three, branches emerge from a conceptacle, and young stages have been observed by us and by previous authors[3,4] growing from the lining wall cells of the conceptacle. This would mean that the gametophytic stage of *Notheia* is entirely endophytic within the conceptacles, and that *Notheia* is essentially a simple or occasionally truly branched plant; the normal *Notheia* plant is actually a compound plant consisting of numerous individuals of possibly several successive generations. Occasional establishment of new *Notheia* plants from *Hormosira* conceptacles might occur by macrozoospores escaping from *Notheia* conceptacles and developing near or inside adjacent *Hormosira* conceptacles, with subsequent fertilization (?) and development of the zygote within the *Hormosira*. The one record of *Notheia* on *Xiphophora* may be a rare chance occurrence.

Notheia is clearly to be classed in the Heterogeneratae group of the brown algae, having different sporophytic and gametophytic generations. The development of the thallus is essentially filamentous, but probably with some true parenchymatous cell formation at older stages. It shows some relationship with certain members of the Chordariales, such as *Spermatochnus paradoxus*[6], which has somewhat similar apical cells and gametophyte, but the only member of this group showing conceptacle formation is the southern hemisphere *Splachnidium rugosum* (which has only one type of sporangium with numerous spores).

Notheia differs from all Chordariales, and in fact from all brown algae, in forming two types of zoospores (if the microzooids are in fact zoospores). Other distinctive features are the three apical cells, the cortex of dichotomous chains of cells with prominent pit connexions, the conceptacles and the endophytic gametophytes and development of new branches. The systematic position of *Notheia* must await knowledge of the full life-history but it certainly forms a distinctive family (Notheiaceae Kuckuck *ex* Schmidt), which may come within the Chordariales or may justify an order of its own.

Brown algae forming zoosporangia normally produce numerous zoospores. The reduction to eight macrozoospores is noteworthy, as well as the production of two morphologically distinct zoospores. *Notheia* may well indicate the possible origin of the Fucales, since were the macro- and micro-zooids to function as gametes (gametophytes being eliminated) and the apical cells become sunk in a depression, the resultant alga would undoubtedly agree well with the Fucales. It is quite possible that the microgametophyte has been eliminated already, and the microzooids function as male gametes; this, however, is still speculation and awaits further examination.

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LETTERS TO THE EDITORS

ASTRONOMY

Light Curves of Meteors

THE luminosity equation for the trail of a meteor is:

$$I = \tfrac{1}{2}\left(\frac{dm}{dt}\right) v^2 \tau \qquad (1)$$

where I is the luminous intensity at any point of the trail, $\left(\frac{dm}{dt}\right)$ is the rate of ablation of the meteoroid at that point, v is the velocity of the meteoroid, and τ is the luminous efficiency factor which determines what fraction of the kinetic energy of the ablated atoms is converted into visible light radiation. It is generally assumed that for bright photographic meteors $\tau = \tau_0 v$, where τ_0 is a constant[1,2]. The luminosity equation therefore becomes:

$$I = \tfrac{1}{2}\left(\frac{dm}{dt}\right) v^3 . \tau_0 \qquad (2)$$

This means that the maximum luminosity of the trail will correspond to the level of maximum rate of ablation of the meteoroid.

In a recent paper, Hawkins and Southworth[3] have pointed out on the basis of their study of a random sample of 360 meteor trails photographed with the Baker Super Schmidt cameras at New Mexico that the observed lengths of the trails are considerably shorter than that predicted by the theory. In one of a series of papers on "The Physical Theory of Meteors", Cook[4] had directed attention to the fact that the early portion of a bright meteor trail studied by him was fainter than predicted by the conventional theory. He worked out an improved theory on the reaction zone model taking into account the thermal radiation from the meteoroid, and found that the modified theory provided a completely satisfactory fit to the observed light curve. Opik[5] has, however, taken exception to the procedure adopted by Cook, and has stated that there is no physical justification for it. In another paper of the series on "The Physical Theory of Meteors", Jacchia[6] has emphasized several anomalies shown by the light curves of faint meteors and has suggested that the theory of progressive fragmentation of the meteoroid along its trail affords a satisfactory explanation of these anomalies.

On the basis of the single-body theory and assuming that $\tau = \tau_0 v$, it can be shown that the equation for the light curve of a meteor is:

$$I = K_1(1 - x)^2 x \qquad (3)$$

where K_1 is a constant for an individual meteor, and $x = \dfrac{p}{p_e} = e^{-h/a}$, where p is the atmospheric pressure at the level corresponding to luminous intensity I, p_e is the pressure at the level corresponding to the end of the trail, h is the height interval between p and p_e and a is the mean scale height of the atmosphere at the levels appropriate to the meteor trail. This equation indicates that, irrespective of the original mass of the meteoroid, the level of maximum luminosity will be at a height of $a \log_e 3$ ($\sim 1{\cdot}1a$) above the level of complete ablation, and that the equivalent radius and mass of the meteoroid at the level of maximum luminosity will be 2/3 of its original radius and 8/27 of its original mass, respectively[1,5].

The equation for the light curve can be normalized by expressing the luminous intensity at any point of the trail in terms of the maximum luminous intensity. The corresponding equation expressed as magnitude difference is:

$$M - M_{\max.} = \Delta M = -2{\cdot}5\left[2\log(1 - x) + \log x + \log\left(\frac{27}{4}\right)\right] \qquad (4)$$

Assuming a scale height value of $a = 6{\cdot}38$ km. the relation between ΔM and h is represented graphically by curve 1 of Fig. 1. This is identical with the theoretical curve in Fig. 5 of the paper of Hawkins and Southworth[3]. Following their procedure, the points corresponding to the beginning and end of the meteor trails have been plotted in Fig. 1 against the appropriate ΔM values. Almost all the points lie within the theoretical curve, showing that the actual lengths of meteor trails are appreciably shorter than what the theory indicates.

I have modified the conventional theory by assuming that τ is not a constant but varies along the trail of an individual meteor, being proportional to the density of the environment at every point of the trail. In this case the equations for I and ΔM become:

$$I = K_2(1 - x)^2 x^2 \qquad (5)$$

$$M - M_{\max.} = \Delta M = -2{\cdot}5[2\log(1 - x) + 2\log x + \log 16] \qquad (6)$$

where K_2 is a constant for an individual meteor and the other symbols have the same meaning as before.

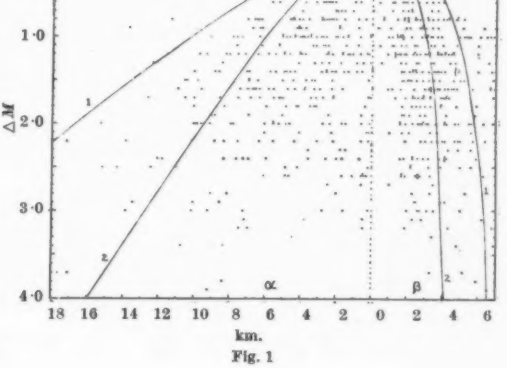

Fig. 1

It can be easily shown that the level of maximum luminosity—this is now different from the level of maximum rate of ablation which remains the same as before—is now at a height of $a \log_e 2 (\sim 0{\cdot}7a)$ above the end of the trail, and that the equivalent radius and mass of the meteoroid at this level will be half its original radius and 1/8 of its original mass, respectively. The relation between ΔM and h is now represented by curve 2 of Fig. 1, which gives a shorter trail for the meteor and the descending branch of which is nearly the median curve through the cluster of points corresponding to the ends of the meteor trails. It will be seen that the modified theory shows better agreement with observation, thus indicating that the interaction between the ablated meteor atoms and the air molecules, which is the primary cause of light emission, has to be explicitly taken into account in the luminosity equation.

The modified theory accounts satisfactorily for the observed light curve of the meteor trail studied by Cook[4]. This meteor appeared at a height of 114 km. at $M = +1{\cdot}9$; it attained maximum luminosity at a height of 84 km. at $M_{max.} = -5{\cdot}7$; it disappeared at a height of 78 km. at $M = -2{\cdot}6$. For the ascending part of the light curve we have, therefore, $\Delta M = 7{\cdot}6$ and $h_b - h_x = \alpha = 30$ km.; for the descending part $\Delta M = 3{\cdot}1$ and $h_x - h_e = \beta = 6$ km. The values of α and β according to the conventional and modified light curves for certain values of scale height are given in Table 1.

Table 1

		α				β		
a (km.)	ΔM	Conventional theory (km.)	Modified theory (km.)	Observed (km.)	ΔM	Conventional theory (km.)	Modified theory (km.)	Observed (km.)
6·38	7·6	50·2	27·2	30·0	3·1	6·4	3·9	6·0
7·00		55·8	30·2			7·1	4·3	
8·00		62·8	34·0			8·0	4·9	

The scale height values considered here are of the correct order as given by the Rocket Panel[7]. We see that the agreement between the modified theory and observation is satisfactory while the conventional theory gives too high values for the length of the meteor trail.

It can be shown that the large scatter of the points corresponding to the beginning of the meteor trails in Fig. 1 and the comparatively smaller scatter of the points corresponding to the end of the trails lend support to Jacchia's fragmentation theory as the cause of meteor anomalies. Fuller details will be published elsewhere.

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GEOPHYSICS

Longitude Effect in Temperate Zone Sporadic *E* and the Earth's Magnetic Field

A recent study[1] of the occurrence of sporadic *E* has shown that the percentage of the time $f_0E_s >$ 5 Mc./s. exhibits a marked longitude effect, having a maximum ($\sim$ 30 per cent) over south-east Asia and minima over South Africa ($\sim$ 3 per cent) and the north-east part of the United States ($\sim$ 6 per cent). That the effect is real, and not due to differences of equipment sensitivity, has been confirmed by very high-frequency forward scatter experiments over the Caribbean and the Philippines[2]. The suggestion was put forward that this longitude effect may depend in a complex manner on both geographical and magnetic latitudes or may be due to meteorological effects[3].

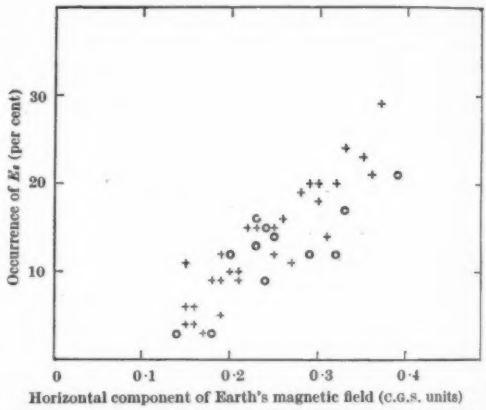

Fig. 1. Plot of percentage of time $f_0E_s > 5$ Mc./s. against horizontal component of the Earth's magnetic field. +, Northern hemisphere; ○, southern hemisphere

The purpose of this communication is to point out that the horizontal component of the Earth's magnetic field has a similar variation at the Earth's surface. In Fig. 1 the percentage of the time $f_0E_s > 5$ Mc./s. for the thirty-one ionosonde stations mentioned by Smith[1] is plotted against the magnitude of the

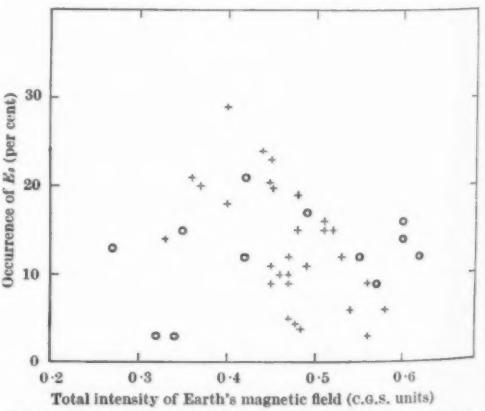

Fig. 2. Plot of percentage of time $f_0E_s > 5$ Mc./s. against total intensity of the Earth's magnetic field. +, Northern hemisphere; ○, southern hemisphere

horizontal component. Results from both northern and southern hemispheres follow the same pattern.

That it is the horizontal component which is correlated with sporadic E appearances is confirmed by plotting the percentage time $f_0E_s > 5$ Mc./s. against the total intensity of the Earth's field (Fig. 2). There is no correlation between the two. There is some correlation between the occurrence of sporadic E and magnetic dip though this is almost entirely due to their correlation with the horizontal component.

It is of interest to inquire which of the various theories put forward to account for sporadic E depend only on the horizontal component of the Earth's magnetic field. Ionization by neutral particles or meteors does not depend on magnetic field at all but ionization by charged particles may be confined to a narrow region in the presence of a magnetic field. The total intensity, however, seems to be of importance here. Movement of existing ionization by winds or electric fields seems to be the most likely source of sporadic E, and is being studied further.

This work is part of the research programme sponsored by the Radio Research Board of the Commonwealth Scientific and Industrial Research Organization.

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Geomagnetic Noise at 230 kc./s.

Geomagnetic radio noise or 'hiss' usually occurs only in the audio region. During severe magnetic storms it has been reported up to 30 kc./s. on a sweep frequency analyser[1] and at 70 and 180 kc./s. on fixed tuned channels[2]. The latter report[2] was written after the records had been destroyed in a fire. New observations at up to 230 kc./s. are described here.

Observations were made simultaneously at the four frequencies 4, 9, 70 and 230 kc./s. (using two dual-beam cathode-ray oscillographs and photographic recording) over the period mid-September to mid-November, 1959. Geomagnetic noise was observed on three occasions only: 2245 U.T., October 31; 0430 U.T., November 3; and around 0330 U.T., November 6. The planetary magnetic index (k_p) ranged from 4 to 6 at these times. The noise burst observed on November 6 is reproduced in Fig. 1. This shows 9 and 230 kc./s. only, since the camera recording 4 and 70 kc./s. jammed on this occasion.

The general appearance of the noise burst—relatively gradual rise and decline of intensity (as distinct from the sharp 'on' and 'off' of man-made interference) and impulsive noise component superimposed on a steady or 'white' noise-level—is typical of geomagnetic noise bursts observed at audio-frequencies. The maximum noise-levels are seen to be 0·8 μV.m.$^{-1}$ (c./s.)$^{-\frac{1}{2}}$ at 9 kc./s. and 16 mμV.m.$^{-1}$ (c./s.)$^{-\frac{1}{2}}$ at 230 kc./s., corresponding to intensities of about 10^{-15} W.m.$^{-2}$ (c./s.)$^{-1}$ at 9 kc./s. and 3×10^{-19} W.m.$^{-2}$ (c./s.)$^{-1}$ at 230 kc./s. The intensities observed at 4 and 9 kc./s. at these times were typical of strong bursts at these frequencies. The 70 and 230 kc./s. intensities were of the same order as previously reported[2]. A striking feature of Fig. 1 is the close correspondence of amplitude fluctuations at the two frequencies. These appear simultaneous to the limit of reading of the time scale ($\sim$ 10 sec.).

A probable mechanism for generation of this noise is Čerenkov radiation from small clouds of charged particles travelling through the ionosphere and exosphere[1,3,6]. Radiation is produced at all frequences for which the velocity of the particle clouds exceeds the group velocity of the radiation (Čerenkov condition). The close correspondence between the two frequency components observed suggests production by the same particle clouds and in the same geomagnetic tube of force since moving charged particles are effectively constrained to a tube of force.

Accordingly the tube of force ending at Hobart (geomagnetic lat. 52° S.) was considered, and the group velocities of 9 and 230 kc./s. radiation call culated[4] as a function of distance along this tube of force. Electron density as a function of radia-distance was obtained from whistler data[5]. The assumption was made that the electron density in the exosphere is spherically distributed. At 9 kc./s. it was found that the group velocity would be everywhere less than 0·12 c. from the ionosphere to the equatorial plane (total distance 15,000 km.). At 230 kc./s. only radiation produced below the gyro-level is observable on the ground. From the ionosphere to the gyro-level (a distance of about 5,000 km.) the 230 kc./s. group velocity would be everywhere less than 0·17 c. Consequently, particle clouds travelling down this tube of force at 0·2 c. (say) could produce Čerenkov radiation at both frequencies. Since the travel-time even of 9 kc./s. radiation produced at 15,000 km. would be less than a second, bursts of radiation at the two frequencies would arrive at the ground simultaneously to our limit of reading.

Estimates of absolute intensities to be expected from the Čerenkov process depend too strongly on

Fig. 1

unknown factors (for example, size and number of clouds and cloud density) to be meaningful. But we can make an order-of-magnitude estimate of the relative intensities based on reasonable assumptions. If these unknown factors were the same for both frequencies we would find that the ratio of intensities produced at 9 and 230 kc./s. would be proportional to the path-length in wave-lengths for which the Čerenkov condition holds[3]. This indicates that the intensity produced at 230 kc./s. should be nearly ten times that at 9 kc./s. However, this ratio will be modified by the ear trumpet effect of the converging tube, giving enhancement proportional to the tube cross-section, which is in turn inversely proportional to the field-strength or gyro-frequency. For the tube of force under consideration this will enhance the ratio in favour of 9 kc./s. by a factor of five. Also if the attenuation in regions of high collision frequency is taken into account (from Helliwell's curves[4]) we find that 230 kc./s. will be attenuated by about eight times as many decibels as 9 kc./s. Agreement with the ratio of intensities observed at Hobart (3×10^3 or 35 db.) is reached for an assumption of about 5 db. for 9 kc./s. and hence about 40 db. for 230 kc./s.

Consideration of tubes of force ending farther south of Hobart require attenuations of 9 kc./s. considerably less than this, in keeping with observations of multi-echo whistler trains. Thus estimates based on this mechanism give reasonable agreement with observations of wide-band noise bursts for frequencies from a few kc./s. to a few hundred kc./s.

It is interesting to note that for this mechanism wide-band bursts will only be observed if the electron density distribution along a line of force is such that the plasma frequency is always greater than the gyro frequency for the range of frequencies observed. Since the electron density in the equatorial plane is obtained from whistler data this gives a lower limit to electron density at each point along the line of force.

My thanks are due to Mr. G. T. Goldstone, who built much of the equipment and assisted in the observations, and to Mr. P. S. Bowling who provided housing for the equipment in the field.

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Hobart, Tasmania. June 10.

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GEOLOGY

The American Oligocene

Eames *et al.*[1] claim that the Oligocene is not represented in the marine sediments of the western hemisphere. This claim seems to conflict with the palæogeographic status of the region.

The Eastern Venezuela Basin may be taken as representative. In Trinidad the Lizard Springs, Navet, Cipero and Lengua formations form a continuous sequence of Palæocene to Miocene age. They are deep-sea deposits, essentially *Globigerina*-oozes, which accumulated along the axis of the basin.

If any appreciable time-interval is not represented in this sequence, its absence must be explained in one of three ways, namely: (1) sedimentation ceased temporarily; (2) sediments were deposited but were eroded subaerially; or (3) sediments were deposited but were removed by subaqueous processes.

Cessation of deep marine sedimentation scarcely merits consideration. The joint accumulation of fine terrigenous material and foraminiferal tests continues in basinal deeps regardless of orogenic and epeirogenic modifications of the peripheral land areas.

A pre-requisite for subaerial erosion would be uplift and emergence of the sea-floor. If this had occurred there would be abundant evidence of orogeny and regional unconformity. Even if the uplift had been epeirogenic, leaving few physical signs of unconformity, the subsidence which followed would leave the unmistakable evidence of shallow marine beds grading up into deep marine marls. The only such interruption of the deep marine sequence in Trinidad is the San Fernando formation, which is firmly correlated with the Upper Eocene (Jacksonian stage) of the region. The upper beds transitional from the shallow marine San Fernando formation to the deep marine Cipero formation are post-Eocene (*G. ampliapertura* zone).

(Eames *et al.* consider the rich Eocene faunas of the San Fernando formation re-worked in the basal Miocene. In more regional terms they imply that the "Jacksonian transgression" was an early Miocene event. The only reason given is their belief that the genus *Pliolepidina* is a Miocene index. It deserves note that in the Eastern Venezuela Basin there are extensive algal-orbitoidal reef limestones which contain *Pliolepidina* in association with such Eocene indices as *Tubulostium*, *Echinolampas*, *Oligopygus* and *Asterocyclina*. These are purely organic limestones with no clastic content except the finest of silt. The *Pliolepidinas* are definitely not re-worked, therefore this genus is unacceptable as a Miocene index in this region.)

Large-scale slumping is recognized throughout the sedimentary column in Trinidad. It is feasible, and even probable, that at any given locality some part or other of the sequence is missing by reason of submarine slumping, but it is scarcely feasible that one particular interval should be missing in this matter over the whole area. Even if this odd situation did exist on the basinal flanks the displaced beds would presumably be recognized in an allochthonous position towards the axis, and their true stratigraphic status would be established by faunal study.

In more regional terms the sequence of Upper Eocene overlain by the *G. ampliapertura* zone is known in a variety of facies ranging from shallow to deep marine. In all cases the same uniform change in the planktonic fauna defines the contact. This implies that, if there is a major hiatus between the two units, it is no more pronounced around the old land masses than in the basinal deeps, a conclusion contrary to the normal precepts of palæogeography. The Eocene top usually falls within a lithologically uniform sequence. Faunally the contact is mainly defined by extinction of a few Eocene species, not by a wholesale change of fauna. No fauna has been recorded which might fit between the suites of the Upper Eocene and the *G. ampliapertura* zone, themselves closely interconnected by evolutionary bonds. It is not realistic to postulate extensive re-working of the Eocene faunas, which are homogeneous except for an appreciable facies-variation consistent with basinal position. This latter remark applies to Trinidad, where the Upper Eocene faunas

are certainly not re-worked in the ordinary usage of that word, though the type San Fernando formation might prove to be an enormous slumped mass. I have studied this interval in Ecuador, Colombia, Venezuela, Trinidad and Barbados in the New World, as well as in Egypt and France in the Old World, and have seen no significant variation from the pattern described. Published records confirm that the same is true in other countries. The one exceptional case is in Antigua, where the *G. ampliapertura* zone rests unconformably on volcanic rocks. The natural conclusion to be drawn is that sedimentation was regionally continuous from the Eocene into the *G. ampliapertura* zone, in which case the latter must be Oligocene.

This palæogeographic reasoning makes regional absence of the Oligocene unlikely in the marine sediments of the western hemisphere. Therefore an alternative explanation would be preferred for any palæontological argument which seems to point to that conclusion. In particular, Eames *et al.* note a new suite of planktonic foraminifera in the Oligocene of East Africa and infer absence of the Oligocene in America because this suite is not known there. An alternative inference is that the new fauna reflects climatic segregation of planktonic suites. Preference of certain species for certain latitudes is well established among the Recent planktonic foraminifera. The marine mid-Tertiaries in the region encompassed by Ecuador, the Gulf Coast States and Trinidad are readily correlated by the planktonic zonation of Bolli (1957) and earlier workers, but both south and north of this tropical belt the scheme tends to break down because the marker-species are scarce or absent. Likewise in the Mediterranean region almost the same zones are recognizable, but attempts to apply the zonation in northern Europe have failed because a different, boreal suite of plankton is present. It seems that a similar situation might well exist in the southern parts of Africa and South America. The observed affinities of this new fauna to Oligocene plankton in Germany and Russia, but apparently not in the Mediterranean countries, give some support to the concept of distinct tropical–subtropical and boreal–austral suites of planktonic foraminifera in the Oligocene.

No detailed comments are offered here on the status of larger foraminifera in this problem, but I opine strongly that the palæogeographical evidence for the presence of the Oligocene in America should outweigh strictly faunal evidence pointing to the contrary. The case of *Pliolepidina*, already cited, shows that like forms were not precisely coëval over the entire world. Regarding the Miogypsinidae, doubt is expressed as to their presence in the *G. ampliapertura* zone as I have never encountered them *in situ* below the *G. dissimilis* zone. Kugler (1954) has referred to them in the older zone, but specifies that he is writing of allochthonous material slumped into Miocene marls. The presence of the ancestral *Rotalia mexicana*-group in the *G. ampliapertura* zone in Ecuador, Colombia, Mexico and Puerto Rico makes the presence of Miogypsinidae at that level improbable.

Extensive bibliographical references to these matters are included in a paper about to be published[2].

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Jusepin, Venezuela.

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Following from Dr. R. M. Stainforth's comments on our communication, we would direct attention to the fact that it contained merely some of the conclusions reached in our study of "Mid-Tertiary Stratigraphical Palæontology", which will be published as an entire, illustrated book, and it is obvious that we could not give the full background in such a short space. However, the following is a very brief outline of part of the picture which has emerged from our studies.

The standard European stages of Upper Eocene, Oligocene and Lower Miocene ages may be traced from the European molluscan facies into the Mediterranean region, where they can be defined in terms of planktonic and benthonic foraminifera; these divisions can be recognized throughout the Middle East to the Far East, where the 'letter-stages' proposed by van der Vlerk for the Tertiary of Indonesia can be readily correlated with the European standard, and both may be correlated with the series of planktonic-foraminiferal zones erected by Bolli and others in the Caribbean. It is an inescapable fact that if the popular age-determinations of Central American middle Tertiary marine sediments are accepted, then that area is palæontologically out of step with the rest of the world. If sedimentation in Trinidad, for example, were really continuous from the Eocene to the Miocene (and the lower Cipero formation be regarded as Oligocene), then one can only conclude that the larger foraminifera (for example, *Miogypsina* s.s.), the planktonic foraminifera (for example, all species of the Globigerinidae which are stratigraphically relevant) and Mollusca (for example, by regional correlation, *Anadara*, *Chione*, *Timoclea*) occur at stratigraphically older horizons in America than anywhere else in the world. As there is no satisfactory evidence to the contrary, we believe that the occurrences of these fossils were isochronous, and that there occurred a major hiatus in the Caribbean sequence, a hiatus including at least the equivalent of the *Nummulites fichteli* beds of Europe ('*c*' and '*d*' stages of the Far East), which contain (in Africa) a rich and distinctive assemblage of planktonic foraminifera, unrepresented in America. We have been able to trace the evolutionary history of many lineages of Globigerinaceae from the Upper Eocene to the Aquitanian in richly fossiliferous and well-dated sediments in East Africa; we have found that the Oligocene stages of these lineages are present in sediments of areas as far removed as western European and Indonesia–Australasia, but that these stages are conspicuously absent in America, between the Jacksonian and the Vicksburgian.

It has been known for decades that the Vicksburg formation rests disconformably on the Jackson, and that it contains in its lower beds (for example, the Red Bluff Clay) abundant re-worked fossils of the Eocene. It has been common knowledge that the sediments of Trinidad, far from constituting a "lithologically uniform sequence" as stated by Dr. Stainforth, have been repeatedly reported to contain boulder beds (with some 'boulders' up to 3,500 cu. ft. in volume), rubble beds with *Pholas*-bored pebbles, and re-worked fossils of Upper Cretaceous, Palæocene, Lower, Middle and Upper Eocene ages immediately above the horizon where we, on quite other (purely palæontological) grounds, would place the Miocene/Eocene unconformity. The San Fernando 'formation' we believe to be the basal bed of the Miocene transgression; far from being "firmly correlated with the Upper Eocene", as Dr. Stainforth suggests, our

world-wide studies suggest that its fossil content is a mixed one, and that its youngest elements are characteristic of the Miocene. This does not conflict with the well-known fact that the lithology of the San Fernando 'formation', surrounding the re-worked boulders, is very close to that of the succeeding Cipero (into which it grades) but quite different from the underlying Navet; it is confirmed by our recognition of the lowest planktonic foraminiferal zones of the Cipero in acknowledged Aquitanian deposits elsewhere in the world.

It should be realized that many other widely held and long-established assumptions fundamental to mid-Tertiary dating in Central America (for example, the supposed Oligocene age of the Antigua Limestone coral fauna), when critically re-examined, prove to have little or no foundation in fact. The ages assigned to some species of several other groups of marine fossils (for example, Echinoidea, Bryozoa, Decapoda, etc.) from the Central American region will need revision in view of the evidence we shall present.

The known stratigraphical distribution of *Pliolepidina* depends on the interpretation of the morphology of that genus; we suspect that Dr. Stainforth has been led astray by W. Storrs Cole's misconceived treatment of *Lepidocyclina* and related genera. We redefine the relevant genera and restrict *Pliolepidina* to forms congeneric with *Multilepidina*, which is not known beneath the Miocene. It should be remembered in this context that no true *Nummulites* have ever been recorded above the Eocene in America, American post-Eocene *Nummulites*-like forms being referable to *Palaeonummulites* (which includes *Operculinella* and *Operculinoides*). With regard to the occurrences of *Echinolampas* and *Oligopygus*, *Echinolampas* ranges from Eocene to Recent, and *Oligopygus* occurs in the Eocene and also at levels at which Lower Miocene planktonic foraminifera occur, so that Dr. Stainforth's implication that they are Eocene indices does not seem wise. We should also like to place on record that the '*Rotalia*' *mexicana* group occurs as high stratigraphically as the Aquitanian in the Mediterranean region, and we believe it to range equally high in Central America. Also, it is mere hypothesis that this group (or even the genus *Neorotalia* to which it probably belongs) was ancestral to any of the Miogypsinidae, and even if this were found to be so, there would be no *a priori* reason to assume that the ancestral group would become extinct at the advent of its descendants.

The references for this study are so extensive, interrelated and mutually inconsistent (any brief selection would be misleading) that we can only refer the reader to the full discussion in our book, which contains a fully comprehensive bibliography, and which will soon be published.

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Major Element Relationships in Tektites

The chemical composition of tektites is a major factor to be considered when investigating theories of tektite origin. Many investigators have remarked on the similarity of the composition of tektites to certain types of sedimentary rocks, and this fact has been held to be indicative of a terrestrial origin. Recent determinations[1] of the abundances of the alkali elements, lithium, sodium, potassium, rubidium and cæsium in australites showed that a close coherence existed between these five elements in different samples; in fact, the concentration of one alkali element was shown to be positively correlated with the concentration of any other to a high degree of significance.

Following on from this, it seemed worth while to investigate the possible correlations between the concentrations of other elements in tektites. This has been done, utilizing the published data for australites. A notable fact emerges, namely, that silica concentration is significantly negatively correlated with the concentrations of aluminium oxide, ferrous oxide, magnesium oxide, potassium monoxide and calcium oxide. By way of illustration we show the silica versus alumina graph (Fig. 1). The regression lines (silica on alumina and vice versa) have been drawn in, and it is interesting to note that both lines pass through 100 per cent silica to within 3 per cent. This fact, plus the presence of the well-known lechatelierite (fused silica) particles in tektites, suggested to us that tektite composition could perhaps be explained on the basis of a mixing process between

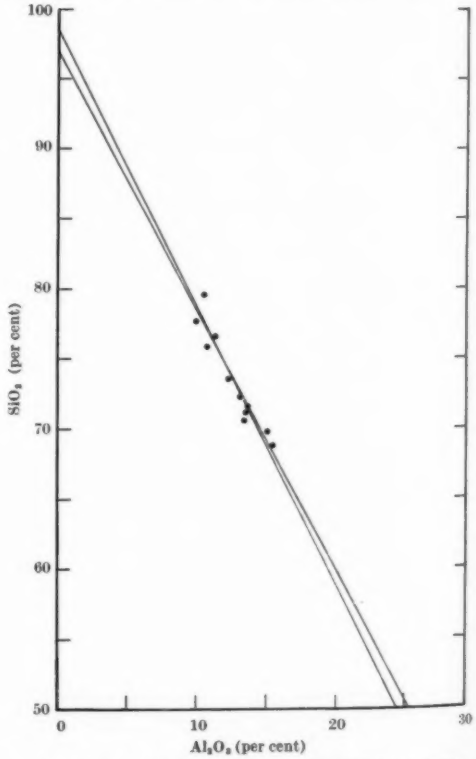

Fig. 1. Inverse relationship between silica (SiO_2) and alumina (Al_2O_3) for the australites. The regression lines are shown

silica and some other (unknown) material. A brief search suggested that the non-volatile (at 1,700° C.) constituents of ordinary shale would form a suitable 'unknown' material, and in Table 1 we give the chemical composition resulting from such a mixing process with a quartz : shale ratio of 1 : 3. Column 2 of the table must be compared with column 3, which gives the average tektite figures. The agreement is seen to be excellent, and it seems evident that a hypothesis that tektites are derived from a shale–quartz mix will explain the major element compositions of tektites. Shale and quartz are both extremely common terrestrial materials, and lack of availability will present no problem. The question of the mechanism of the mixing process remains. In this connexion we can present no evidence, though the Urey comet hypothesis[2] still seems to us to be a promising theory.

Table 1. Comparison of Composition of Tektites with that of a Mixture of SiO_2 with the Non-Volatile (at 1,700° C.) Constituents of Shales

	1	2	3
SiO_2	64·32	73·24	73·73
TiO_2	0·72	0·54	0·86
Al_2O_3	17·05	12·79	12·47
FeO	6·72	5·04	4·92
MgO	2·70	2·03	2·12
CaO	3·44	2·58	2·50
Na_2O	1·44	1·08	1·32
K_2O	3·59	2·69	2·32
Total	99·98	99·99	100·24

Column 1. Composition of average shale from Pettijohn[3] (Table 61, p. 344) recalculated to 100 per cent following removal of water, carbon dioxide, SO_3 and organic matter.
Column 2. Composition of a mixture of three parts of column 1 with one part of silica.
Column 3. Average composition of 61 tektites from Barnes[4]. The analyses of Darwin glass and Libyan Desert glass quoted by Barnes[4] are excluded from this average.

The above results will be published in greater detail elsewhere, and discussed more fully.

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PHYSICS

A Comparison of Atomic Beam Frequency Standards

Standard frequencies obtained from various cæsium atomic beam frequency standards have been compared in a number of instances[1–4]. The results of Holloway *et al.*[1] showed agreement to about 2×10^{-10} between the commercial beam standards developed and manufactured by the National Company and the atomic standard at the National Physical Laboratory, Teddington, England[4].

Two dissimilar beam standards at the National Bureau of Standards have been compared over the past several months. Their frequencies agree to within $\pm 1{\cdot}5 \times 10^{-11}$ (standard deviation of the mean for the comparisons and estimated uncertainty due to effects of pertinent parameters).

These machines employ Ramsey-type excitation, the separation of the oscillating fields being 55 cm. in one case and 164 cm. in the other. Beam dimensions are 0·003 in. × 0·100 in. and 0·015 in. × 0·187 in., respectively. The hot wire detectors (20 per cent iridium–platinum alloy) are used in conjunction with conventional electrometer circuits. Typical signal-to-noise ratios range between 100 and 400. The uniform magnetic C field in the transition region is produced by passing a d.c. current of about 1 amp. through a conducting section of a cylindrical brass tube contained within a magnetic shield. A double shield is used on the longer machine and a single shield on the shorter.

There are a number of uncertainties in absolute frequency measurements introduced by the measuring devices themselves. Those effects contributing most significantly to the overall uncertainty of the measurements are: (*a*) the magnitude and non-uniformity of the C field; (*b*) a phase difference between the two oscillating electromagnetic field regions; (*c*) a lack of purity of the electromagnetic field exciting the atomic transition.

The magnitude of the C field was determined in four different ways: by calculation from the known geometry of the conducting strip and the current used; by direct measurement using a rotating-coil fluxmeter sensitive to 0·002 oersted; by measurement of the low-frequency $\Delta F = 0$, $\Delta m_F = \pm 1$ transitions which are strongly dependent on the magnitude of the field; and by measurement of other microwave transitions, such as the $(F = 4, m_F = 1) \leftrightarrow (F = 3, m_F = 1)$ transition, which are more sensitive to the field than the standard frequency transition. The uniformity of the C field was determined by measuring the low-frequency transitions, utilizing small coils located at each end of the resonant cavity and at the centre of the transition region. These localized field measurements indicate that the maximum C field variation along its length is $\pm$ 0·002 oersted. This non-uniformity can introduce at most an uncertainty of 4×10^{-12} in the measured frequency. At the time of the initial comparisons of the two standards the values of the C field magnitude as measured by the different methods agreed to within the precision of the measurements ($\pm$ 0·002 oersted) for both machines. Since that time, however, the shielding properties of the mumetal shields have deteriorated to some extent, accompanied in the case of the longer machine by a discrepancy among the various types of field measurements of about 0·004 oersted at a field of 0·080 oersted. In order to reduce the resulting uncertainty in the frequency measurements to below 1×10^{-11}, smaller C fields (about 0·020 oersted) have been used in the more recent comparisons. Possible frequency shifts arising from the nearness of other transitions at these low fields have been calculated and found to be well below 1×10^{-11} for the degree of symmetry of position and amplitude observed for the nearby transitions.

The oscillating fields exciting the $(F = 4, m_F = 0) \leftrightarrow (F = 3, m_F = 0)$ transition are produced at the two ends of a single resonant U-shaped rectangular cavity operating in the $TE_{0,\,1,\,60}$ mode for the shorter machine and in the $TE_{0,\,1,\,100}$ mode for the longer machine. The cavities were precisely electroformed for symmetry about the coupling hole to the

microwave transmission line from the frequency multiplier chain. The atomic beam grazes the shorted end walls of the U-shaped cavity. Provision for tuning the cavities in the event of large room-temperature changes is provided by a tuning plunger opposite the coupling hole.

Frequency uncertainties introduced by a phase difference between the two ends of the cavity were investigated by rotating the cavity 180°, that is, by interchanging the two oscillating field regions. No frequency shift was measurable under this operation for either machine.

If the electromagnetic field exciting the transition is not pure, that is, if this signal is frequency modulated, rather large frequency uncertainties are possible in the measurements. The exciting radiation is ordinarily produced by frequency multiplication by a factor of 1836 from a stable quartz crystal oscillator. Side-bands in the power spectrum introduced in the oscillator or first stages of the frequency multiplier chain are enhanced significantly by the multiplication process. What is worse, side-bands resulting from frequency modulation are not, in general, symmetrically placed about the 'primary' signal. The power spectrum of the exciting radiation for the Bureau standards was examined using an ammonia maser stabilized frequency multiplier chain as a spectrum analyser[5]. The signal was found to have a bandwidth of about 6 c./s. at 9,000 Mc./s., was symmetrical and contained no observable side-bands. Any shift introduced from this source would be much less than the precision of measurement. In general, however, side-bands will be present to some extent. In one experiment where side-bands were deliberately introduced, the measured frequency was shifted by 3×10^{-9}.

The effects discussed above are those that we consider to contribute most significantly to the uncertainty of absolute frequency measurements if adequate care is not taken in construction and testing. We have found it possible to reduce these uncertainties to a level below 1×10^{-11}.

Initial comparisons of the two standards were made during a ten-day period with each daily comparison consisting of an average of from 15 to 25 measurements with each standard. If $\Delta\nu_i$ denotes the zero-field frequency difference between the two machines for the ith day, then the measured average difference for the ten-day period was:

$$<\Delta\nu>_{av} = \frac{1}{n}\sum_{i=1}^{n} \Delta\nu_i = 0, \text{ where } n = 10$$

The standard deviation of the mean for this particular test was:

$$\sigma_M = \frac{1}{\nu}\left[\frac{\sum_{i=1}^{n} (\Delta\nu_i - <\Delta\nu>_{av})^2}{n(n-1)}\right]^{1/2} = \pm 1{\cdot}6 \times 10^{-11}$$

The estimated uncertainties introduced by the effects discussed above fall within this precision. During later tests on the standards a more complete set of comparisons was obtained consisting of 18 separate comparisons taken over a period of one month. The average zero-field frequency difference for this period was 9×10^{-12} with a standard deviation of the mean of 6×10^{-12}. To this standard deviation was added the maximum uncertainty in frequency contributed by the C field (9×10^{-12}), giving $\pm 1{\cdot}5 \times 10^{-11}$ as the limits quoted for the agreement of the two machines. The uncertainty introduced by the discrepancy in the C field measurements noted above was reduced to less than 1×10^{-11} by using low fields. Since this discrepancy seems to be associated with a noticeable deterioration of the C field shielding, it is likely that for most precise results the shields will have to be exchanged periodically for newly annealed ones.

One indication of the reproducibility of measurements made with the longer machine was obtained by comparing eight separate sets of measurements of 'Atomichron 106' during an 8-hr. period. Each set or sub-group consisted of 15 measurements taken in a period of about 15 min. The standard deviation of the mean for each sub-group was 1×10^{-11}, while the standard deviation of the mean for the average of all the results was 5×10^{-12}. A similar experiment was conducted in which an extremely low-drift crystal oscillator with its crystal immersed in liquid helium was measured instead of the 'Atomichron'. These measurements gave 7×10^{-12} as the standard deviation of the mean for each sub-group and 2×10^{-12} as the standard deviation of the mean for the average of all the results taken during the period of several hours. The various parameters affecting the frequency measurements, then, are sufficiently constant that measurements can be made to a precision of 2×10^{-12} in periods of several hours or longer; however, uncertainty in the exact values of some of the parameters limits the accuracy to about $1{\cdot}5 \times 10^{-11}$.

The longer of the two machines is the present United States Frequency Standard. The shorter machine is an alternate standard[3]. The frequency assumed for the $(F = 4, m_F = 0) \leftrightarrow (F = 3, m_F = 0)$ transition of cæsium in zero field is 9192631770·0 c.p.s. The best comparison between cæsium and Ephemeris Time at present is that given by Markowitz, Hall, Essen and Parry as 9192631770 ± 20 c.p.s.[6].

Corrections for the 60 kc./s. standard frequency broadcasts of Station *WWVB* (formerly *KK2XEI*), Boulder, Colorado, are made each week and are available upon request.

We believe that the experiments demonstrate that with adequate care in construction and testing, atomic beam standards can be expected to agree in frequency without special recipes in design, and indeed they behave precisely as one would predict from theory—one need only know the values of the pertinent parameters to sufficient accuracy. This information must be obtained from appropriate tests.

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Isotopic Analysis of Silicon by Solid-Source Mass Spectrometry using Negative Ions

An A.E.I. mass spectrometer, type *M.S.2/S.*, fitted with an electron multiplier and having a triple tungsten filament as source for the thermal ionization of solids is being used for measurements of the abundance of silicon isotopes.

The instrument normally records the amplified current peaks of positive ions. In the case of silicon, it has not been possible so far to detect a positive ion from thermal ionization, but after reversing the polarity of the accelerating voltage and magnetic field of the instrument, negative ions have been measured corresponding to SiO_2^- at values of m/e of 76, 77 and 78.

The filament source is loaded with an alkali silicate, and the best results are obtained from cæsium silicate. This is in accordance with the ionization potential of cæsium, which is the lowest for the natural alkali element series; thus no ions are obtained from sodium silicate and only small peaks from potassium silicate.

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High-Energy Oxygen Ions in Carbon Dioxide Mass Spectra

Mass spectra of carbon dioxide have been recorded using a low accelerating voltage and magnetic scanning. Under these conditions, a variant of those due to Mohler *et al.*[1], ions with more than thermal energies will appear as satellite peaks in the spectra. The energies of the ions can then be derived from the separation of the peaks by the use of the mass spectrometer focusing equation.

A high-energy state in oxygen ions has been observed as a satellite on the high-mass side of the main peak due to thermal energy oxygen ions. The energy of this state in oxygen ions was found to be 3·5 V. when the energy of the ionizing electrons was 70 V. The apparent appearance potential was 35 V. (the voltage scale being corrected by the known value for the thermal energy peak of the oxygen ion[2].

These values would suggest that the ions were due to the dissociation of doubly charged ions[1]; those possible are O_2^{2+}, CO^{2+} or CO_2^{2+}. It will be seen that the appearance potential is lower than those found for O_2^{2+} from oxygen (50 V.) and for CO^{2+} from carbon monoxide, namely, 42 V. (ref. 2). Ions of O_2^+ were not detected under the present conditions; and thus if the mechanism is $O_2^{2+} \rightarrow 2\,O^+$, a previous reaction similar to one suggested by Ogryzlo and Schiff[3], namely, $CO_2 + O \rightarrow CO + O_2$, seems preferable to a rearrangement reaction involving O or a secondary reaction induced by pressure. The CO_2^{2+} mechanism, namely, $CO_2^{2+} \rightarrow CO^+ + O^+$, is the most probable, as CO_2^{2+} ions exist in the mass spectrum and the appearance potential of the O^+ ion state is comparable to that of CO_2^{2+} ions, about 36 V. by comparison with the known value for A^{2+} ions[2]. This reaction was also postulated by Newton[4] to explain other phenomena in the mass spectra of carbon dioxide. However, if the reaction is indeed $CO_2^{2+} \rightarrow CO^+ + O^+$, a definite energy-state would be expected in the CO^+ ions.

Only the main ion peak, together with high-energy tailing, was apparent in the C^+, CO_2^{2+} and CO^+ ion peaks. The main excitation energy found for O^+ ions from carbon monoxide under these conditions (70 V. ionizing electrons, 230 V. accelerating voltage) was 2·4$_2$ V., compared with Hagstrum's value of 1·5 V. by the retarding potential method and Berry's value of 0·65–2·5 V. by the deflexion method[2]. When ionizing electrons of 70-V. energy were used, the O^+ ion peak from oxygen showed a high-energy tail but no evidence of a discrete high-energy state.

The ratio between the ions of high-energy state and the thermal energy ions was affected by variations in the ion repeller voltage, which altered the time of residence of the ions in the ion chamber. Low voltages, down to 0·5 V., favoured the high-energy state, presumably because the time available for dissociation was increased, whereas higher voltages, up to 8 V., resulted in the predominance of the thermal-energy ions.

The mass spectrometer is a 10-cm. radius 90° deflexion A.E.I. instrument with a source of Nier type.

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METALLURGY

Formation of Fayalite in Roast Reduced Jaspilite

A magnetizing roasting treatment of a fine-grained hæmatite-quartzite known as jaspilite, occurring in South Australia, is at present being evaluated[1]. When heated in reducing gases at appropriate temperatures, 550–650° C., the hæmatite in jaspilite is converted to magnetite. Extensive microcracking also occurs in the quartz matrix (Fig. 1*A*). This cracking allows freer access of reducing gases to the hæmatite particles undergoing reduction and facilitates grinding prior to magnetic concentration[2].

In contrast to prior investigations[2], roast reduction treatments much above 650° C. have led to lower rates of reduction, lower recoveries of magnetite, and less apparent cracking.

One reason for this, other than the effect of increased temperature in reducing the tendency of quartz to crack and part from the hæmatite, has been found to be the formation of fayalite ($2FeO.SiO_2$), by a solid-state diffusion reaction at the operating temperature.

That fayalite will form when hæmatite is reduced in the presence of silica at higher temperatures, 800–1,000° C., has previously been reported[4,5]. Indeed,

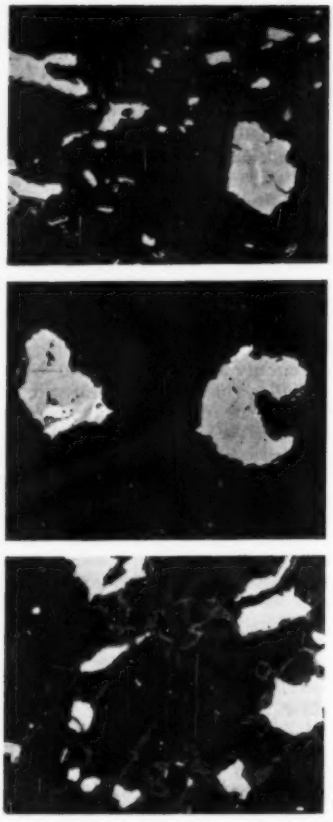

Fig. 1. Roast reduced jaspilite, vacuum mounted in epoxy resin, polished and examined, unetched, in reflected light : *A*, reduced in hydrogen at 550° C. for 10 min. Magnetite (grey) in a matrix of cracked quartz (dark grey) (× *c.* 330); *B*, reduced in 4 per cent carbon monoxide, 6 per cent carbon dioxide and 90 per cent nitrogen at 900° C. for 160 min. Fayalite forms at the magnetite-quartz, not at the hæmatite-quartz interfaces (× *c.* 660); *C*, reduced in hydrogen at 950° C. for 45 min. Over-reduction to wüstite and fayalite. The fayalite fills cracks in the quartz. The resulting voids near the wüstite have been filled with mounting resin (× *c.* 330)

Darken[6] has shown that, in the presence of an excess of silica, fayalite, not magnetite, becomes the stable phase under the reducing conditions normally employed.

This present work has shown that fayalite, formed at as low a temperature as 650° C., has the capacity to bond the magnetite and quartz, sealing the interface (Fig. 1*B*) and thus, by hindering access of reducing gases to the interior reduction zone, decreases the rate of reduction. Fayalite was identified by X-ray diffraction and by refractive index measurements.

The dihedral angle of fayalite in quartz has been found to be low, and at higher temperatures fayalite diffuses into, and fills, the microcracks (Fig. 1*C*). Non-magnetic fayalite adhering to silica and magnetite adds to the difficulties in effecting high recoveries of a high-grade concentrate in subsequent grinding and magnetic separation.

Further details of this work, including a discussion of the kinetics and mechanism of reduction cracking, will be published elsewhere.

The permission of the director of research, Dr. H. K. Worner, to publish this communication is gratefully acknowledged.

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Stabilization of Austenite by Hydrogen

Attempts to study the possible effects of hydrogen on the austentite–martensite transformation in steels have always to reckon with the difficulty of retaining adequate amounts of hydrogen in them, regardless of the amounts initially present. The austenitizing and heat treatment of steels are generally carried out at virtually atmospheric pressures or in vacuum. As the diffusion-rates of hydrogen at elevated temperatures are considerable, the amount of hydrogen retained in the austenite, prior to its being heat-treated, is generally small. It is also not so much in excess of the solubility limits as to render it likely that the hydrogen atoms play a part similar to that played by carbon atoms, which are generally retained in appreciable amounts and in a state of considerable supersaturation when the matrix changes to the body-centred structure. In the comparatively small amounts, therefore, in which hydrogen is retained in normally austenitized and heat-treated steels the likelihood of its affecting the stability of austenite may well be negligible (Ramachandran, E. G. and Dasarathy, C., unpublished work).

If, however, either a mode for austenitization and subsequent heat treatment of a steel with any specified, high amount of hydrogen can be devised, so as to retain adequate hydrogen in the austenite, or the hydrogen content of a normally heat-treated steel is increased when it is still austenitic and at a stage prior to its transformation to martensite, it may be possible to detect the effects of hydrogen on the austenite–martensite transformation. As austenitization in hydrogen at such high pressures as to ensure its adequate retention is, however, difficult to carry out (there is no guarantee even then, that hydrogen is not nearly completely lost by the austenite when it is cooled in salt, lead, oil or aqueous baths to transform to martensite) the first course does not appear to be feasible. The failure, therefore, by Ko and Edmondson[1] to detect any stabilization of austenite in a nickel steel, even when it was cathodically impregnated with hydrogen at room temperature but transformed after austenitization, 24 hr. after the impregnation, at 1,100° C. for 30 min. in vacuum, may well be understood.

The second alternative is quite feasible and allows the effect of hydrogen, when suitably introduced, on the stability of austenite to be studied, as may be

seen from the following results, obtained on a 1·1 per cent carbon, 2·83 per cent chromium steel and a 18-8 low carbon (0·08 per cent) unstabilized stainless steel.

A. Chromium steel, austenitized in argon at 980° C. for 1½ hr. M_s temperature, 93° C. (approx.)

Heat treatment	Hardness (V.P.N., 30 kgm. load) (Mean of four impressions)
1. Quenched from 980° C. in boiling water at 98° C., held for 90 sec., and quenched in an aqueous solution of 10 per cent sulphuric acid, held at 18° C.	755
2. Quenched from 980° C. in an aqueous solution of 5 per cent sulphuric acid held at 98° C. for 15 sec., during which, using the bath as electrolyte, the specimen was cathodically impregnated, transferred to boiling water at 98° C. for 75 sec. and afterwards quenched in an aqueous solution of 10 per cent sulphuric acid, held at 18° C.	710
3. Quenched from 980° C. in an aqueous solution of 5 per cent sulphuric acid held at 98° C., subjected thrice to hydrogen impregnation and removal cycle as in 2 and finally quenched in an aqueous solution of 10 per cent sulphuric acid, held at 18° C.	710

B. Stainless steel, austenitized in argon at 1,050° C. for ½ hr. Approximate M_s temperature, −130° C.

Heat treatment	Hardness (V.P.N., 30 kgm. load) (Mean of four impressions)
1. Quenched from 1,050° C. in water at 18° C.	155
2. Quenched from 1,050° C. in water at 18° C., held at room temperature for 40 min., quenched in liquid air (−192° C.) and upquenched to water at 18° C.	169
3. Quenched from 1,050° C. in water at 18° C., transferred to an aqueous solution of 5 per cent sulphuric acid and cathodically impregnated for 40 min., quenched in liquid air and upquenched to water at 18° C.	164
3*a*. Above specimen aged at room temperature for 60 hr.	162
4. Quenched from 1,050° C. in water at 18° C., cathodically impregnated for 40 min., as in 3, transferred to boiling water at 98° C. for 75 sec., quenched in liquid air and upquenched to water at 18° C.	158

The above stabilization of austenite by hydrogen and the conditions under which it is detectable can be theoretically worked out from the concepts regarding austenite stabilization put forward originally by Cohen *et al.*[2], and amplified by us[3,4] and will be discussed in a subsequent communication.

We thank Dr. B. R. Nijhawan, director of the National Metallurgical Laboratory, Jamshedpur, for discussion and permission to publish this communication.

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Retained Austenite and the Tempering of Martensite

The decomposition of retained austenite as the second stage in the tempering of quenched steels is well recognized, and has been explored extensively. However, the role of retained austenite in the first stage tempering reaction has not been studied widely. That this may not be a passive role is suggested by the infrequent reports that a change in austenite chemistry by carbon enrichment accompanies this stage of tempering[1–3].

The first-stage tempering reaction takes place within the martensite platelets and occurs quite rapidly without the need for an induction period[4,5]. This process appears to occur discontinuously by the precipitation of ε carbide and the creation of a low-carbon martensite containing approximately 0·25 per cent carbon[3–5]. That is, the original supersaturation of the martensite is not relieved completely during the first-stage reaction. Normally, it is not until the third stage of tempering that the supersaturation of this low-carbon martensite is reduced further by the precipitation of the more stable carbide–cementite.

The reason for a matrix carbon content in the 0·2–0·3 per cent carbon range has received two divergent interpretations based on metastable equilibrium concepts. One view[4,5,7] ignores any interaction between martensite and retained austenite and assumes that the matrix carbon content is dictated by a metastable equilibrium between ε carbide and the low-carbon martensite. Alternatively[3], the matrix carbon content may be determined by a metastable equilibrium between ferrite (low-carbon martensite) and supercooled (retained) austenite. The latter concept is based on extrapolation of the ferrite (*GP*) and austenite (*GS*) solubility curves on the Fe—C equilibrium diagram. In effect, these two interpretations represent alternate means whereby the supersaturation of martensite may be lowered. In this sense, they compete with the more stable equilibrium involving cementite, and kinetic factors will determine the relative importance of each of the three reactions in the overall decomposition sequence. The part played by the ε carbide and cementite reactions is recognized clearly as the first and third stages of the tempering process; however, interaction with the retained austenite requires further clarification.

One of the critical points in deciding between these two interpretations of the matrix carbon content centres around the extrapolation of the ferrite and austenite solubility lines. It seems likely that these curves will exhibit retrograde behaviour[3] so that the solubility of carbon in ferrite (in metastable equilibrium with austenite) should be much less than 0·25 per cent carbon. Under this condition, it must be presumed that the 0·25 per cent carbon-level is controlled by metastable equilibrium with ε carbide. Nevertheless, when a martensite plate is surrounded by austenite, this low-carbon martensite should transfer much of its excess carbon to the austenite in an effort to achieve the lower carbon content demanded by the ferrite–austenite equilibrium. Thus, it should be possible to distinguish between these two interpretations of the matrix carbon content by studying the influence of retained austenite on the first stage tempering reaction.

According to this concept, martensite plates surrounded by austenite should lower their carbon content (below 0·25 per cent carbon) much more rapidly than those surrounded by other martensite plates. To check this possibility, two samples of a 9395 steel (composition: 0·95 per cent carbon, 0·22 per cent silicon, 0·60 per cent manganese, 3·07 per cent nickel, 1·23 per cent chromium, 0·13 per cent molybdenum) were prepared with different austenite–martensite ratios. Both samples were quenched in water after 24 hr. at 2,400° F. This produced 40–50 per cent martensite. One of these

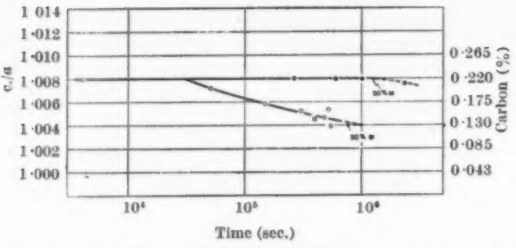

Fig. 1. Percentage of carbon in martensite as a function of holding time at 270° F.

samples was cooled immediately in liquid nitrogen which increased the amount of martensite to 90 per cent. The oscillating crystal technique of Kurdyumov and Lysak[5] was employed to determine the carbon content of the martensitic matrix after appropriate tempering treatments at 270° F. Fig. 1 illustrates the influence of retained austenite on the carbon content of the matrix of tempered martensite.

In this steel, the first stage tempering reaction initially yields a matrix composition of 0·22 per cent carbon regardless of the amount of retained austenite. However, the matrix carbon content of crystals containing 50 per cent martensite was lowered below this level much more rapidly than that of crystals containing 90 per cent martensite. X-ray diffraction studies demonstrated that virtually no additional transformation of austenite accompanied these tempering treatments and that the retained austenite was enriched significantly in carbon. Thus, it is apparent that austenite does act as an effective sink to drain the excess carbon from the martensitic matrix.

The partition of carbon from martensite to austenite indicates that the composition of the low-carbon martensite must be attributed to the metastable equilibrium involving ε carbide rather than the one involving retained austenite. In fact, it seems likely that re-solution of ε carbide helps to maintain the carbon-levels at the values reported in Fig. 1. Furthermore, this interpretation is consistent with the observation[10] that steels containing less than 0·2 per cent carbon do not undergo the normal first stage tempering reaction involving the precipitation of ε carbide.

The partition of carbon from martensite to austenite may contribute significantly to property changes associated with tempering. Generally, this has not been recognized in the analysis of reaction curves obtained by measuring property changes such as electrical resistivity, change in length, evolution of heat, etc. It is apparent that tempering is more complicated than the current theoretical treatments predict, so that a re-examination of tempering kinetics in the light of this and other phenomena may provide a deeper and more satisfying understanding of the theory of tempering processes.

These results also have an important bearing on the bainitic mode of austenite decomposition. The presence of ε carbide in low-temperature bainite[6] suggests that this transformation product passed through a decomposition sequence closely related to that in the formation and tempering of martensite. However, the demonstration that the matrix of low-temperature bainite contains approximately 0·1 per cent carbon rather than 0·25 per cent carbon appears to deny this relationship and suggests that bainite may bypass the tetragonal state characteristic of martensite[7].

This apparent anomaly is removed by the results presented in Fig. 1. Since bainite plates grow slowly[8], partition of carbon from bainite to austenite can accompany the growth process. In this light, it is suggested that low-temperature bainite forms initially (at the advancing tip of the plate) as a supersaturated ferrite with a carbon content which may not differ greatly from that of the parent austenite. Much of this supersaturation is lost rapidly by the precipitation of ε carbide which locally could result in a matrix carbon content in the 0·2–0·3 per cent carbon range. However, as growth of the plate continues, the excess carbon can escape to the surrounding austenite in much the same way as that demonstrated for the tempering of martensite in the presence of large amounts of retained austenite.

Thus, the low carbon content of the matrix of bainite is quite consistent with a supersaturated ferrite mechanism for the mode of formation of bainite.

We wish to express our appreciation to the Office of Naval Research, U.S. Navy, for support of this work.

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CHEMISTRY

Recombination of Oxygen Atoms in the Gas Phase

The rate of gas phase recombination of oxygen atoms at 10° C. in a flow system has been measured. The experimental method was essentially that described by Schiff[1]. Oxygen atoms were produced from electrolytic gas containing a small percentage of water vapour by passage through a microwave discharge. The decay of oxygen atom concentration along the axis of a cylindrical 'Pyrex' tube, 4 cm. in diameter and 100 cm. long, was followed by a movable isothermal probe similar to that used by Schiff[1]. Linear flow-rates in the range 60–250 cm./sec. and total pressure of 0·3–2 mm. mercury were employed. The initial atom concentration, estimated from the power input to the probe, was of the order 5–10 per cent.

The decay process was found to be first-order in oxygen atoms, but second-order in oxygen molecules.

This conclusion is in agreement with that of Schiff and supports his proposed mechanism:

$$\begin{aligned} O + O_2 + O_2 &\rightarrow O_3 + O_2 \qquad k_1 \\ O + O_3 &\rightarrow 2O_2 \qquad k_2 \\ O &\rightarrow \tfrac{1}{2} O_2 \text{ wall} \qquad k_3 \end{aligned}$$

If a stationary state is assumed for $[O_3]$, then:

$$\frac{-d[O]}{dt} = [O]\left\{2k_1[O_2]^2 + k_3\right\}$$

The theoretical model employed by us in evaluating k_1 and k_3 assumes one-dimensional flow and includes axial diffusion but neglects radial diffusion (on the grounds that the radial concentration gradient is probably very small[2]).

For a first-order reaction, the equation of continuity for the reacting species for a steady state is:

$$-D\frac{d^2n}{dx^2} + V_0\frac{dn}{dx} + k_0 n = 0 \qquad (1)$$

where D is the binary diffusion coefficient, V_0 is the linear flow speed, n is the concentration of the reactive species and k_0 is the rate constant for the reaction. This equation neglects the small change in V_0 over the reaction zone. The solution of (1) subject to the boundary conditions:

$$n = 0 \text{ at } x = \infty,\ n = n_0 \text{ at } x = 0$$

is

$$n = n_0\, e^{\frac{-kx}{V_0}}$$

where

$$k_0 = k\left(1 + \frac{kD}{V_0^2}\right) \qquad (2)$$

k/V_0 is thus the slope of the conventional log n versus distance plot shown in Fig. 1. For the proposed mechanism $k_0 = (2k_1[O_2]^2 + k_3)$. When $\frac{kD}{V_0^2} \ll 1$, $k \simeq k_0$. Plots of $kv[O_2]^2$ and $k_0v[O_2]^2$ are shown in Figs. 2 and 3. Fig. 2 shows a distinct deviation from linearity, which is largely eliminated in Fig. 3, in which a value of $D = 2{\cdot}69 \times 10^2/p$ (p = pressure in mm. of mercury) has been used. This value of D has been estimated by Andrussov[3] on a theoretical basis.

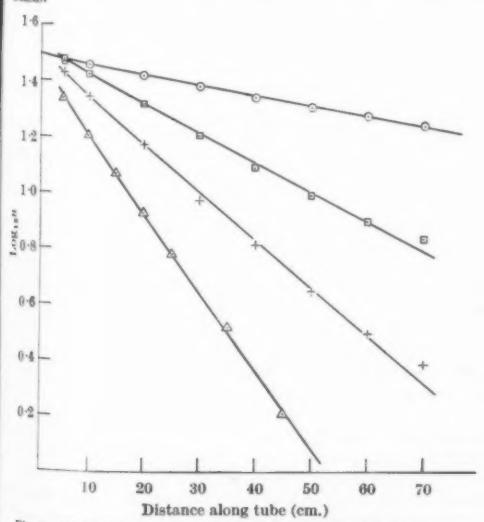

Fig. 1. Plot of log n versus distance for different total pressures. ○, $p = 0{\cdot}335$; ⊡, $p = 0{\cdot}625$; +, $p = 0{\cdot}797$; △, $p = 1{\cdot}015$ mm. mercury

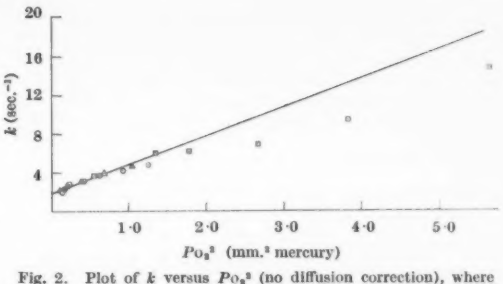

Fig. 2. Plot of k versus Po_2^2 (no diffusion correction), where the mass flow-rates of oxygen are as follows: △, $3{\cdot}19 \times 10^{19}$ molecules/sec.; ○, $3{\cdot}82 \times 10^{19}$ molecules/sec.; ⊡, $4{\cdot}87 \times 10^{19}$ molecules/sec.

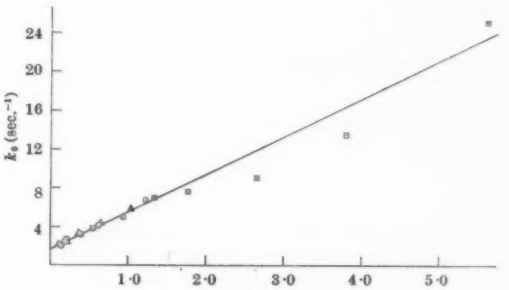

Fig. 3. Plot of k_0 versus Po_2^2 (diffusion correction made). The oxygen flow-rates are as for Fig. 2

The values of k_1 and k_3 obtained from the data incorporated in Fig. 3 using equation (2) were:

$$k_1 = 6{\cdot}0 \times 10^{14} \text{ c.c.}^2 \text{ mole}^{-2} \text{ sec.}^{-1}$$
$$k_3 = 1{\cdot}7 \text{ sec.}^{-1}$$

The value of k_3 can be readily converted into a dimensionless recombination coefficient $\gamma = 2k_3R/\bar{c}$ (where R is the radius of the tube and $\bar{c}$ is the mean speed of the atoms), whence $\gamma = 1{\cdot}1 \times 10^{-4}$.

If no correction for diffusion were made and k_0 were put equal to k (as was done by Schiff), then the corresponding approximate values from the data in Fig. 2 would be:

$$k_1 = 4{\cdot}6 \times 10^{14} \text{ c.c.}^2 \text{ mole}^{-2} \text{ sec.}^{-1}$$
$$k_3 = 1{\cdot}9 \text{ sec.}^{-1}\ (\gamma = 1{\cdot}2 \times 10^{-4})$$

Schiff found $k_1 = 1{\cdot}0 \times 10^{14}$ c.c.^{2} mole^{-2} sec.$^{-1}$ and $\gamma = 0{\cdot}77 \times 10^{-4}$.

A recent investigation of the kinetics of this reaction followed by paramagnetic resonance has been made by Strandberg *et al.*[4]. If their data are analysed on the basis of the theoretical model used here, the reaction appears to be roughly first-order with k in the range 22·0–38·0 sec.$^{-1}$, the total oxygen pressure being 5 mm. mercury. Correcting for diffusion according to equation (2), k_0 is found to be in the range 27–42 sec.$^{-1}$. Using Strandberg's quoted values of $\gamma = 3{\cdot}2 \times 10^{-4}$, $R = 0{\cdot}5$ cm., k_1 is evaluated by us as $0{\cdot}6$–$1{\cdot}8 \times 10^{14}$ c.c.^{2} mole^{-2} sec.$^{-1}$.

Other recent values of k_1 reported in the literature are: 2×10^{14} (Kaufmann[5]); $0{\cdot}79 \times 10^{14}$ (Benson and Axworthy[6]); $\sim 10 \times 10^{14}$ (Steinberg *et al.*[7]).

A comprehensive theoretical treatment of the appropriate flow problem, including the effects of

viscosity, pressure gradients, axial and radial diffusion and chemical reaction, is extremely difficult to handle, but the simplified model presented here appears to describe the experimental situation fairly adequately in this case. It appears important to us that some correction for axial diffusion along these lines should be made when the quantity $\frac{kD}{V_0^2} > 0{\cdot}1$.

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Use of Reflectance Measurements in assessing the Colour Changes of Ageing Bloodstains

It is well known that the colour of bloodstains changes from red to brown with increasing age; but the rate at which these changes occur and the factors which influence this rate are not so well established[1-3]. This is probably in part due to the use of subjective visual assessment of the changes. Extraction of the bloodstains and subjecting the extracts to spectrophotometric analysis suffers from the defect of being destructive of the stain and is sometimes complicated by simultaneous extraction of dye and dirt from the stained object. There is also evidence that the extraction methods may accelerate the colour changes (Kind, S. S., unpublished results).

The value of being able to assess the age of bloodstains, even within wide limits, can on occasion be useful. For example, a person accused of assault may allege that bloodstains present on his clothing are his own, of long standing, and resulted from a cut while shaving. Age assessment may help here if grouping tests prove of no assistance.

The development of photo-electric colorimeters of high sensitivity and precision, the readings of which can be readily converted to the C.I.E. system of colour specification[4], offers a method of non-destructive testing, based on the surface colour of the stain, and free from any subjective judgment.

To test this view, stains were prepared as follows. Sufficient fresh venous blood was collected from six male individuals (age-range 17–45 years) to cover an area rather greater than 4 cm. × 2·5 cm. in the middle of a Whatman No. 1 filter paper circle. When dry the circle was then cut exactly to 4 cm. × 2·5 cm. to eliminate differential shrinkage of the paper which otherwise made it difficult to press the stained area flat without any wrinkling. The samples were then fixed with small pieces of adhesive tape to the back of a black metal mask with an aperture 3·0 cm. × 2·0 cm. cut in it. Marked into the metal of the mask was a rectangle of exactly the same size as the stained paper so that the samples could be placed in exactly the same position on the 'Colormaster' differential colorimeter on each occasion that their colour was measured. Over the back of the samples and the mask a piece of black velvet fixed to black 'Perspex' was placed. This served to exclude light reflected from the back of the samples and to keep them flat.

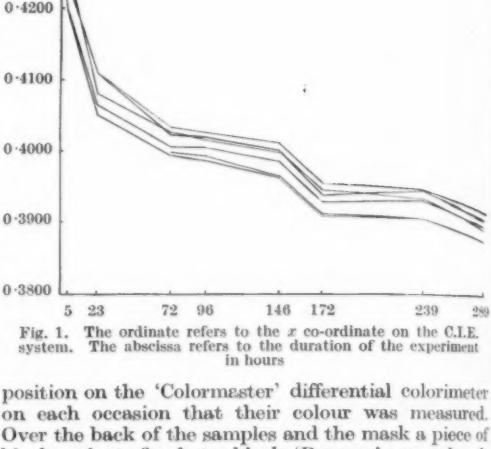

Fig. 1. The ordinate refers to the x co-ordinate on the C.I.E. system. The abscissa refers to the duration of the experiment in hours

The C.I.E. chromaticity co-ordinate x obtained for the six stains is presented in graphical form (Fig. 1). The chromaticity co-ordinate y showed little change over the period of the experiment apart from a slight but steady increase (mean 0·3280 increasing to 0·3330) and the luminance factor after an initial small drop in the first 24 hr. remained constant. The precision of a single reading was estimated by taking 12 successive sets of readings of the red, green and blue reflectance values on one bloodstain, the sample being removed and replaced on the mask between each set. The means and standard deviations obtained are shown in Table 1.

Table 1

	Mean reflectance (per cent)	Standard deviation
Green	13·046	0·013
Red	17·752	0·018
Blue	8·832	0·011

This shows very high precision quite adequate for the purpose in hand.

From Fig. 1 it can be seen that the changes in colour can best be followed from the x chromaticity co-ordinate and that the greater part of the change takes place in the first three days. Afterwards the rate of change is small, and is such that the daily change would only be detectable by a trained observer who had both the changed and unchanged colour before him at one time. It therefore seems unlikely that it is possible to tell the age of bloodstains by visual means alone with any certainty.

The conditions of temperature, humidity and lighting under which the stains are kept doubtless have an effect on the rate of colour change. In these experiments the stains were kept in the dark at room temperature (about 18° C.) between measurements. The use of high-precision colorimetry would seem to offer a basis for preparing master curves of colour changes against time for different environmental

conditions and thus by measuring the rate of change of the colour of an unknown sample to estimate its age by extrapolation. Not enough results are, however, available from the present investigation to enable this to be done.

I wish to thank Mr. S. S. Kind, of the Home Office Forensic Science Laboratory, Harrogate, for helpful discussions.

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Determination of Fatty Acids of Lower Molecular Weight by Gas Chromatography

Analytical methods for the qualitative and quantitative analysis of fatty acids have been concerned primarily with the fatty acids of higher molecular weight because of their importance in health and nutrition. Small quantities of fatty acids as the methyl esters can be separated by the gas-liquid partition chromatography of James and Martin[1,2]. The fractionation is based on the length of the carbon chain with the more recent development, including fractionation on the basis of unsaturation[3–5]. These methods, however, do not provide for the convenient analysis of the acids of lower molecular weight in a mixture of fatty acids. By modification of the method of Craig and Murty[5] it is possible to separate and identify the fatty acids of lower molecular weight with the same column as that used for the separation of the acids of higher molecular weight.

An 'Aerograph' gas chromatographic instrument, model *A*-100, was employed for the fractionation of the methyl esters of the fatty acids. The stainless steel column, ¼ in. outer diameter and 10 ft. long, was packed with 'Chromasorb', 60–80 mesh (Johns-Manville, Celite Division, New York), impregnated with polyester butanediol succinate[5]. 5 gm. of the polyester dissolved in 200 ml. of acetone were added to 25 gm. of 'Chromasorb'. The acetone was removed by heating on a steam-bath, after which the mixture was dried in an oven at 110° C. for 6 hr. A filament current of 210 m.amp. was maintained on the standard 'Aerograph' four-filament detector. A 'Varian' 10 mV. recorder was modified with a range selector switch to give a 1 mV. full-scale sensitivity.

The original procedure was modified for the separation of the lower molecular weight esters. A known mixture of fatty acids, consisting of acetic, propionic, *iso*butyric, butyric, 2-methyl butyric, *iso*valeric, valeric, *iso*caproic, caproic, enanthic, and caprylic acids, were converted to the corresponding methyl esters by reaction with diazomethane, using the microtechnique of Roper and Ma[6]. Since these esters would be characterized by lower boiling points, the column was maintained at a temperature of 92° C. with helium used as the carrier gas at a flow-rate of 40 ml. per min.

A chromatogram showing the separation of these lower molecular weight fatty acid esters is shown in Fig. 1. Using this technique, it is possible to separate the lower molecular weight fatty acids in approximately 28 min.

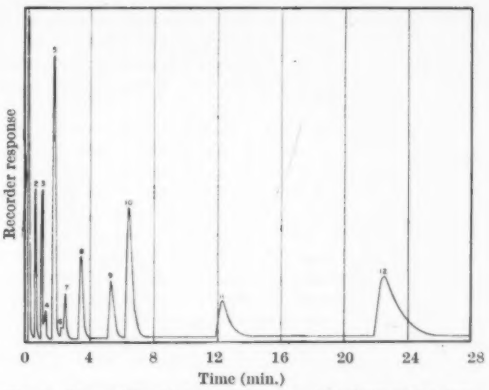

Fig. 1. Separation of lower molecular weight fatty acid esters. 1, Solvent (diethyl ether); 2, methyl acetate; 3, methyl propionate; 4, methyl *iso*butyrate; 5, methyl butyrate; 6, methyl-2-methylbutyrate; 7, methyl *iso*valerate; 8, methyl valerate; 9, methyl *iso*caproate; 10, methyl caproate; 11, methyl enanthate; 12, methyl caprylate. Efficiency: 3,320 theoretical plates (methyl caprylate)

The separation of the higher molecular weight fatty acids can be accomplished by raising the temperature to 216° C. and increasing the flow-rate to 60 ml. per min. The column is brought to equilibrium and another sample injected into the column.

By comparing the peak area of an ester such as methyl caprylate which appears on both chromatograms it is possible to establish a quantitative relationship between the lower and higher molecular weight fatty acids. If no peak overlaps, the addition of a known amount of methyl caprylate will establish a reference point.

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BIOCHEMISTRY

Microbiological Transformation of Terpenes: Hydroxylation of α-Pinene

The microbiological transformations of steroids and alkaloids have opened up a new line of approach towards the synthesis of many biologically active compounds. Relatively little is known, however, about the capability of micro-organisms to transform the simple terpenoid compounds. Recently, Bradshaw *et al.*[1,2] have studied the microbiological transformation of camphor.

The yields of essential oils from some oil-bearing plants are known to be considerably enhanced if the plants are infected by micro-organisms[3]. The present communication deals with the microbiological transformations of α-pinene by moulds.

A preliminary screening of fungi on Czapek–Dox medium enriched with yeast-extract, corn-steep liquor and 0·5 per cent α-pinene (I) resulted in the selection of a strain of *Aspergillus niger* for further studies. A 24-hr. shake culture of this strain metabolized 0·5 per cent pinene in 4–8 hr.

In a typical fermentation the mould was grown in shake cultures for 24 hr. *d*-α-Pinene (I) was added to the culture (0·5 ml. per 100 ml. of the medium) and the fermentation was continued for 4 hr. (The purified sample of α-pinene employed in these experiments had a rotation of $[\alpha]_D^{25}$ + 24·5°. This rotation would correspond to a mixture of 70 per cent *d*- and 30 per cent *l*-α-pinene.) After this period a second lot of pinene (0·5 per cent) was added and the fermentation was allowed to proceed for another 4-hr. period. The culture was filtered. The mycelial pellets were extracted with a little acetone and the acetone extract was added to the culture filtrate, which was then extracted first with ether (extract *A*) and then with butanol (extract *B*). Extract *A* was washed free from acids by aqueous sodium carbonate and evaporated to yield a pale yellow oil. Unreacted pinene was removed from this oil by a countercurrent distribution between hexane and 90 per cent aqueous ethanol. The ethanolic layers containing the oxygenated conversion products of pinene were carefully evaporated. The oily residue yielded on distillation, under reduced pressure, a clear, colourless oil and a semi-crystalline residual mass.

The distillate was further fractionated into an alcohol (II) and a ketone (III) by bisulphite treatment. The residue in the distillation flask, as well as the butanol extract (extract *B*), yielded a crystalline diol (IV) on chromatography over alumina.

Alcohol II: b.p. 140° (bath temp.)/16 mm. $[\alpha]_D^{25}$ + 64° (alcohol); molecular formula $C_{10}H_{16}O$ (found, C, 78·62, H, 10·6); infra-red absorption 3,420 cm.$^{-1}$ (OH). It gave a crystalline *p*-nitrobenzoate, m.p. 90° $[\alpha]_D^{25}$ + 14° (alcohol).

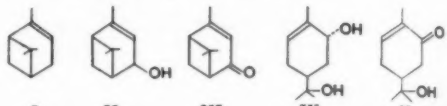
On mild chromic acid oxidation in pyridine or by oxidation with solid manganese dioxide in chloroform the alcohol (II) yielded the ketone (III) in nearly quantitative yields.

Ketone (III): b.p. 130° (bath temp.)/14 mm. $[\alpha]_D^{25}$ + 223° (ethanol); molecular formula $C_{10}H_{14}O$ (found, C, 79·82, H, 9·51); ultra-violet absorption $\lambda_{max.}$ 253 mμ ($\varepsilon_{max.}$ 6,480); infra-red absorption 1,665 cm.$^{-1}$ (conjugated C=O) and 1,615 cm.$^{-1}$ (double bond). It formed a semicarbazone m.p. 203–205°.

From these properties the ketone (III) was identified as *d*-verbenone. For comparison *d*-verbenone was synthesized according to the method of Badoche[4]. A comparison of the physical properties, ultra-violet and infra-red spectra of verbenone and its semicarbazone with those of the ketone (III) and its corresponding derivative conclusively established the identity of compound III. There was no depression in the mixed melting point of the semicarbazones. The alcohol (II) could, therefore, be characterized as *cis d*-verbenol on the basis of its optical rotation and the melting point and the optical rotation of its *p*-nitrobenzoate[5].

Diol (IV): m.p. 144–145° $[\alpha]_D^{25}$ + 152° (ethanol); molecular formula $C_{10}H_{18}O_2$ (found, C, 70·66; H, 10·7); strong infra-red absorption at 3,260 cm.$^{-1}$ (OH). It was identified as *d*(+)-*trans*-sobrerol on the basis of the following properties:

It showed no depression in the mixed melting point with synthetic *trans* sobrerol prepared by acid-catalysed rearrangement of α-pinene epoxide[6]. The physical properties and the infra-red spectra of (IV) were identical with those of the synthetic product. Both were converted to the identical ketone, hydroxy-carvotonacetone (V) ($\lambda_{max.}$ 232 mμ, $\varepsilon_{max.}$ 9,200), on oxidation with manganese dioxide.



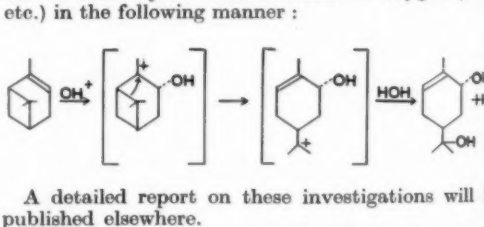
I α-Pinene II *cis*-Verbenol III Verbenone IV *trans*-Sobrerol V Hydroxy-carvotonacetone

Under optimum conditions the yields of these products on the basis of the pinene utilized were 20–25 per cent verbenol, 2–3 per cent verbenone and 2–3 per cent sobrerol. The major fraction of the pinene metabolized appears to have been broken down to smaller fragments and probably oxidized to carbon dioxide and water.

Verbenone, verbenol and sobrerol have been identified among the auto-oxidation products of α-pinene. The major product of auto-oxidation is, however, verbenone with only small amounts of the alcohol, whereas verbenol is the major metabolite obtained by microbiological oxidation. Moreover, in the biological process these products were obtained in optically pure form, presumably from *d*-α-pinene.

The formation of *cis*-verbenol may be accounted for either by a free-radical attack at the allylic position, or by removal of a hydride ion from the same position through the mediation of an electrophilic species, such as an incipient OH^+.

d-trans-Sobrerol may be presumed to be derived from *d*-α-pinene by a postulated attack on the 1,2 double bond by an electron deficient oxygen (OH^+, etc.) in the following manner:



A detailed report on these investigations will be published elsewhere.

We wish to express our thanks to Drs. S. C. Bhattacharyya and V. Jagannathan for their interest in this work and helpful suggestions.

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Excretion of Catechol after Ingestion of Quinic and Shikimic Acids

THE detection of catechol in acid-hydrolysed normal human urine and cow's urine was reported recently by von Euler and Lishajko[1]. The origin of the catechol was not known. The excretion of hippuric acid in the urine of man after oral ingestion of calcium quinate was reported by Lautemann in 1863[2]. It occurred to us that if an aromatic ring can be formed in the animal body from quinic acid (1 : 3 : 4 : 5-tetrahydroxy*cyclo*hexane carboxylic acid), then urinary catechol might have a similar origin. Further, an exogenous (dietary) precursor is implicated since we are unable to detect catechol in the urine of rats fed a highly purified diet. Quinic and shikimic acids are known to be present in edible plants.

Adult albino rats were fed a purified diet (sucrose, casein, salts, oil, vitamins) and given quinic acid by stomach tube (100 mgm./rat). A 48-hr. urine sample was collected and subjected to two-dimensional paper chromatographic analysis. The lower phase of a chloroform/acetic acid/water (2 : 1 : 1) mixture was used in the first direction followed by 20 per cent potassium chloride in the second direction. Each developed chromatogram was air-dried and carefully examined under short-wave ultra-violet light and then sprayed with diazotized sulphanilic acid followed by 20 per cent sodium carbonate. Thus the positive identification of a urinary metabolite was based on its R_F values in two dimensions, its behaviour under ultra-violet light and its colour with diazotized sulphanilic acid–sodium carbonate.

In our chromatographic system[3] hippuric acid is located at R_F 0·60 × 0·87, gives no colour with the diazotized sulphanilic acid reagent and absorbs ultra-violet light. The only notable deviation from normal in the case of the ether extract of the acidified urine of rats given quinic acid by stomach tube was a marked increase in the size of the area corresponding to hippuric acid. Catechol was not detected. Similarly, catechol was not detected after acid hydrolysis (2 *N* hydrochloric acid reflux, 30 min.) of the ether extract, suggesting that this fraction does not contain an ether-soluble conjugate of catechol. Finally, the ether-extracted urine was acid-hydrolysed and re-extracted with ether. When this ether extract was examined chromatographically, catechol was readily detected at R_F 0·50 × 0·74. Catechol absorbs ultra-violet light and when sprayed with the diazotized sulphanilic acid reagent undergoes a very characteristic colour change from red to green to blue-black. Another characteristic of catechol is its tendency to darken on an unsprayed chromatogram, presumably due to spontaneous oxidation.

When shikimic acid was administered to rats by stomach tube (100 mgm./rat) the same results were obtained as described above for quinic acid. Thus catechol was clearly present on the chromatogram of the acid-hydrolysed ether-extracted urine. In addition, an unidentified metabolite of both quinic and shikimic acids having the same chromatographic characteristics as vanillic acid was detected in the urine after hydrolysis.

Rats were also fed quinic acid mixed in the purified diet at a level of 3 per cent. Catechol was readily detected free (unconjugated) as well as conjugated in the urine. According to Garton and Williams[4], catechol is formed and excreted principally as a mono-glucuronide or monosulphate derivative in the urine of rabbits receiving benzene by stomach tube.

It is difficult to estimate the amount of catechol excreted in urine, due to its instability, especially when subjected to acid hydrolysis. A minimal amount of 2 mgm. of catechol was excreted during a period of two days by two rats ingesting a total of 1·5 gm. of quinic acid in the diet.

Whether aromatization of quinic and shikimic acids to form catechol is dependent on intestinal micro-organisms is not known. Ayengar *et al.*[5] reported that "extracts of rabbit-liver acetone powder catalysed the formation of catechol from 3,5-*cyclo*hexadiene-1,2-diol", but not from D-quinic acid. Mitoma *et al.* isolated an enzyme system from guinea pig liver which was able to aromatize *cyclo*hexanecarboxylic acid[6].

From these results it is tempting to conclude that the catechol in human and cow urine may be formed from quinic acid and related substances in the diet.

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Staining for Iodine in Chromatograms of Human Plasma : an Artefact due to Thiourea or Thiouracils

UNTIL the report by Werner and Block[1], it was generally thought that at most only very small quantities of iodinated tyrosines are present in normal human serum. This conclusion was based on ^{131}I-chromatographic studies using plasma extracts from subjects given radioiodine. Werner and Block report, however, that when the iodine distribution in normal human serum is analysed chemically by the ceric–arsenite reaction[2], approximately half the circulating iodine is present in the form of mono-iodotyrosine or di-iodotyrosine, although the radio-activity in the same chromatogram is distributed according to the results of other workers. Hitherto these results have been very difficult to understand, and they are inconsistent with the rapid deiodination seen when iodotyrosines are injected[3]. We have recently confirmed Werner and Block's results indicating a discrepancy between the distribution of stable and radioactive iodine, when using very similar methods to estimate the distribution of iodinated amino-acids in human serum. Our extraction and chromatographic techniques differed from those of Mandl and Block[4] only in that the acidified serum was extracted three times with butanol and that the chromatogram was unidimensional and run in acid-butanol / 2 *N* acetic acid (1 : 1). We find that when

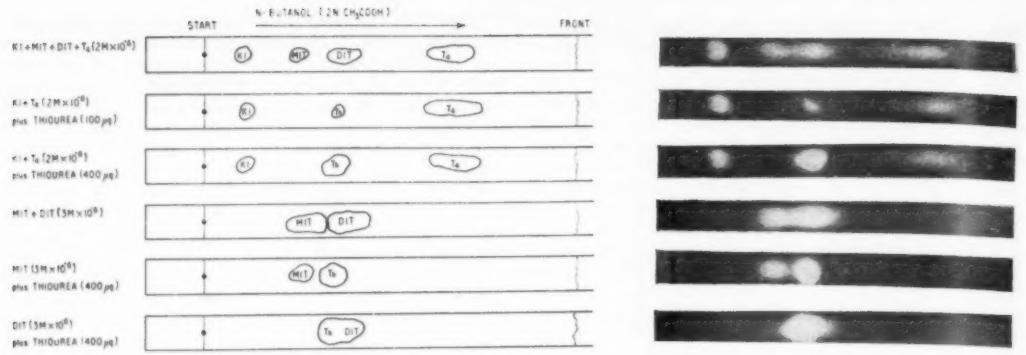
n-BUTANOL (2N CH3COOH)
START
FRONT
KI + MIT + DIT + T4 (2M×10⁻⁶)
KI + T4 (2M×10⁻⁶) plus THIOUREA (100 μg)
KI + T4 (2M×10⁻⁶) plus THIOUREA (400 μg)
MIT + DIT (3M×10⁻⁶)
MIT (3M×10⁻⁶) plus THIOUREA (400 μg)
DIT (3M×10⁻⁶) plus THIOUREA (400 μg)

Fig. 1. Chromatograms stained for iodine by the ceric–arsenite reaction: influence of thiourea on iodotyrosine positions

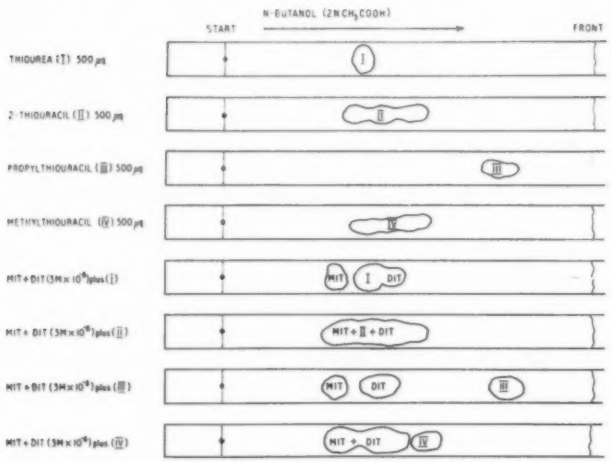
n-BUTANOL (2N CH3COOH)
START
FRONT
THIOUREA (I) 500 μg
2-THIOURACIL (II) 500 μg
PROPYLTHIOURACIL (III) 500 μg
METHYLTHIOURACIL (IV) 500 μg
MIT + DIT (3M×10⁻⁶) plus (I)
MIT + DIT (3M×10⁻⁶) plus (II)
MIT + DIT (3M×10⁻⁶) plus (III)
MIT + DIT (3M×10⁻⁶) plus (IV)

Fig. 2. Behaviour of some thiouracil derivatives on chromatograms stained for iodine by ceric–arsenite

the chromatogram of serum collected 48 hr. after a 100 μc. dose of iodine-131 is scanned for radioactivity little or no counts are recordable in the mono- or di-iodotyrosine positions, whereas when the same chromatograms are evaluated chemically for iodine by the ceric–arsenite reaction, iodotyrosines appear to be present in considerable quantity.

However, activation analysis by neutron bombardment in the Medical Research Council cyclotron to produce iodine-128 did not reveal any iodine in the region of the chromatogram corresponding to the positions of mono- and di-iodotyrosine. 5–10 ml. of normal serum had been run on paper from which the spots were cut. Evaluated by the fading with ceric–arsenite the chromatogram showed an iodine distribution of 20·5 per cent as iodide, 8·5 per cent as mono-, and 19·6 as di-iodotyrosine, whereas activation analysis produced iodine-128 from the iodide spot but less than 0·2 μgm. iodine/100 ml. serum from either of the apparent iodotyrosine spots (the thyronine region was not tested).

Further work suggests that the staining in the mono- and di-iodotyrosine regions of the chromatogram developed with ceric–arsenite may be due to the presence of thiourea. This substance had been added in μgm. quantities at all stages of the chemical processing in order to prevent any artefacts from the oxidation of iodide to iodine. In our chromatographic system thiourea, 2-thiouracil or methyl thiouracil has recently been found to run in the position of either mono- or di-iodotyrosine or between them, depending on the presence or absence of the iodotyrosines (Figs. 1 and 2); propylthiouracil runs in the position of the thyronines.

Careful study by direct colorimetry of the effect of thiourea on the time-course of the ceric–arsenite reaction shows that although there is some rapid initial fading this is virtually imperceptible when small (less than 100 μgm.) quantities of thiourea are used. Furthermore, the subsequent rate of fading is almost indistinguishable from that given by iodide, especially with small thiourea concentrations (Fig. 3).

From this we conclude that the apparent presence of mono- and di-iodotyrosine on chromatograms stained for iodine when prepared from normal human serum by our initial procedure may be an artefact due to added thiourea. Preliminary studies with activation analysis and an improved chromatographic technique have as yet failed to reveal detectable amounts of iodotyrosines in normal fasting human serum. Thus we cannot confirm the conclusions of Werner and Block, and we suggest that the apparent iodotyrosine staining which they find may have been

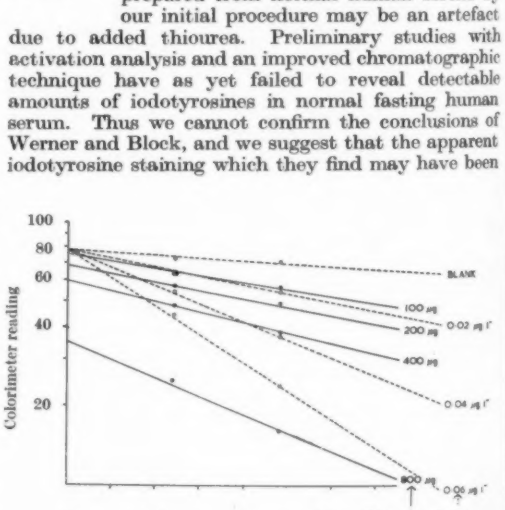

Fig. 3. Effect of thiourea on the time course of the ceric–arsenite reaction

an artefact due to thiouracil added during their extraction procedure.

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* Supported by a grant from the Wellcome Trust.

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Inhibition of Glucuronosyl Transferase by Progestational Agents from Serum of Pregnant Women

Glucuronide conjugates are formed by the condensation of an alcohol or acid aglycone with uridine diphosphoglucuronic acid by the enzyme, glucuronosyl transferase[1]. A functional immaturity of this enzyme system and perhaps a deficiency of uridine diphosphoglucuronic acid has been observed in new-born animals[2]. Since indirect-reacting bilirubin is converted to bilirubin glucuronide by means of glucuronosyl transferase[3], Billing *et al.*[4] have suggested that physiological hyperbilirubinæmia of the new-born infant might be the result of a deficiency of this enzyme system. Recently, Lathe and Walker[5] reported inhibition of bilirubin conjugation in liver slices by serum of pregnant women. This communication reports studies aimed at identifying the inhibitor substances and assessing their importance in hyperbilirubinæmia of the new-born.

The effect of a final serum concentration of 14 per cent upon *o*-aminophenol conjugation by rat liver homogenate was studied using the method of Levvy and Storey[6]. The results (Fig. 1) indicated inhibition of 64·0 ± 21·8 per cent, 42·8 ± 22·5 per cent, and 48·2 ± 20·0 per cent by serum from pregnant women, immediately *post-partum* women, and new-born infants respectively, in contrast to inhibition of 1·3 ± 1·0 per cent and 26·8 ± 9·8 per cent by serum from adult males and adult non-pregnant females. Analyses of serial determinations indicate a gradual increase of *o*-aminophenol inhibition as term is approached, and a rapid fall after delivery.

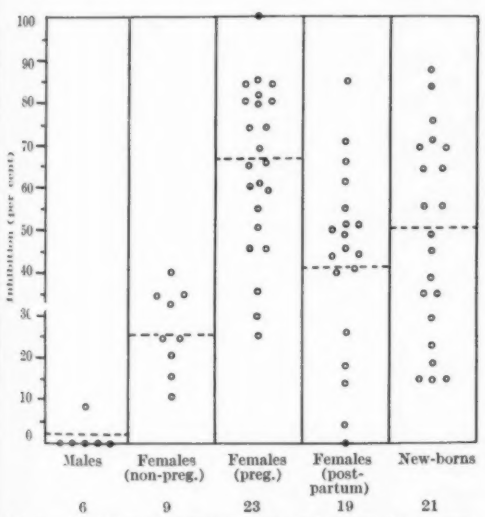

Fig. 1. Inhibition of *o*-aminophenol conjugation by various types of sera. Incubation mixture consisted of: 0·5 *M tris* buffer, pH 8·0, 0·1 ml.; 0·5 *M* magnesium chloride, 0·1 ml.; $1 \cdot 1 \times 10^{-2}$ *M* uridine diphosphoglucuronic acid, 0·1 ml.; $1 \cdot 25 \times 10^{-3}$ *M* *o*-aminophenol in 0·01 *M* ascorbic acid, 0·2 ml.; 10 per cent rat liver homogenate, 1·0 ml.; and serum, 0·22 ml. Incubation was carried out at 37° C. for 30 min.

The neutral lipids extracted from one litre of pooled serum from pregnant women were chromatographed on a silica column using gradient elution with benzene–ethyl acetate[7]. Four fractions were obtained which resulted in inhibition of *o*-aminophenol conjugation. The fraction showing most of the inhibitory activity contained a material which was chromatographed on propylene glycol-treated paper using toluene as developer; its migration corresponded to pregnanediol-3*a*,20*a*. On repeated recrystallization from ethanol, hexagonal prisms were obtained which showed a change in crystal structure at 210–220° C. and melted at 239·5–240° C. (Köffler micro-melting point). Authentic pregnanediol-3*a*,20*a* melted at 239·5–240° C. and a mixed melting point with the isolated crystals was 237·5–238·5° C. Infrared spectra of the isolated material and pregnanediol are identical. On the basis of migration on paper and colour tests, other fractions are thought to contain *allo*-pregnanetriol and pregnanolone. All three compounds were found to inhibit conjugation *in vitro*.

Table 1. Inhibition by Progestins of Phenolphthalein Conjugation by Rat Liver Microsomal Fraction

Compound	Final concentration (γ/ml.)	Inhibition (per cent)
17*a*-Ethyl-19-*nor*testosterone	0·8	50
Progesterone	6·0	50
Pregnanediol	4·0	30
17*a*-Ethinyl-19-*nor*testosterone	10·0	36
Androstenedione	8·0	27
17*a*-Ethyl-Δ^{5-10}-19-*nor*androstanediol	12·0	27
17*a*-Ethinyl-Δ^{5-10}-19-*nor*androstanediol	18·0	27
17*a*-Hydroxyprogesterone	16·0	15
17*a*-Methallyl-19-*nor*testosterone	32·0	50

Each flask contained microsomes equivalent to 0·3 gm. liver, 760γ phenolphthalein, 400γ uridine diphosphoglucuronic acid, 0·05 *M tris* buffer at *p*H 7·6, 0·012 *M* magnesium chloride, 0·015 *M* nicotinamide, 0·06 *M* potassium chloride, and is incubated at 37° C. for 30 min.

With these findings, it was decided next to test a series of progestational agents of physiological importance. 17α-ethyl-19-*nor*testosterone, progesterone, pregnanediol, 17α-hydroxyprogesterone, and 17α-methallyl-19-*nor*progesterone, 17α-ethinyl-19-*nor*testosterone, 17α-ethyl-Δ^{5-10}-19-*nor*androstanolone, 17α-ethinyl-Δ^{5-10}-19-*nor*androstanolone, 17α-hydroxyprogesterone, in order of decreasing effectiveness, were found to inhibit glucuronide formation as shown in Table 1.

With progesterone, inhibition was found using *o*-aminophenol, phenolphthalein, and bilirubin as substrates in both homogenates and microsomal fractions prepared from rat and guinea pig liver.

These observations may help to explain the jaundice which has been observed with prolonged administration of 17α-ethyl-19-*nor*testosterone and other steroids. High levels of progestins in maternal serum during pregnancy could play a part in the physiological hyperbilirubinæmia of the new-born.

This investigation was aided by grants from the Association for the Aid of Crippled Children, the U.S. Public Health Service (*A*–2222) and the Smith, Kline and French Foundation. We are indebted to Dr. E. G. Daskalakis for the authentic pregnanediol-3*a*,20*a* and for help with the paper chromatography, and to Eva Gawronska Kot and Dorothy Poppish for technical assistance.

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Plant-Growth Activity of α-Alkylphenylacetic Acids and 1,2-Benzo-1-*cyclo*alkene-3-carboxylic Acids

1,2,3,4-Tetrahydro-1-naphthoic acid (1,2-benzo-1-*cyclo*hexene-3-carboxylic acid) was found to have a high plant-growth activity. Its lower homologue, 1-indancarboxylic acid (1,2-benzo-1-*cyclo*pentene-3-carboxylic acid) was reported later to be considerably active though less than the former[1]. In the homologous series of these two acids, the 1,2-benzo-1-*cyclo*alkene-3-carboxylic acids, the spatial relation of the carboxyl group to the ring system is influenced by the alicyclic ring structure. On the other hand, in the α-alkylphenylacetic acids, which may be regarded as the 'open forms' of the above acids, it is unnecessary to consider such an influence on the carboxyl group. In order to examine the effect on plant-growth activity of such a difference in the structure, we tested activities of these series of compounds in the pea straight-growth test.

In the α-alkylphenylacetic acids, the terminal of the alkyl chain is considered to lie preferably far from both the benzene ring and the carboxyl group in order to reduce steric repulsions to a minimum. The chain of *n*-alkanes has been shown by Bonham and his co-workers[2] to exist predominantly in all-*trans* configuration or in those which involve at least one *gauche* conformation. Therefore, the spatial relation of the carboxyl group to the ring may not change appreciably, even if the length of the side-chain changes. The carboxyl group in this series emerges considerably from the plane of the benzene ring. Fitting of the molecule to the site of action may, however, be interfered with when the molecular dimension increases due to elongation of the side-chain. The molecular dimensions of *n*-butylphenylacetic and *n*-amylphenylacetic acids, which are inactive, may be too large for fitting.

In the 1,2-benzo-1-*cyclo*alkene-3-carboxylic acids, increasing the carbon atoms in the alicyclic ring may not affect the molecular dimension so remarkably as in the above-mentioned series, because of their 'condensed ring structure'. The spatial configuration of the carboxyl group, on the other hand, may be affected considerably. Judged from the ultra-violet spectra and the molecular model, the carboxyl group of benzo*cyclo*butene- and indan-carboxylic acids takes a fixed conformation rising considerably from the plane of the benzene ring. The carboxyl of tetrahydro-naphthoic acid, which is more active than the two lower homologues, emerges from the ring plane more conspicuously when it takes a quasi-axial conformation, one of the two conformations inferable. The carboxyl group of benzo-*cyclo*heptene- and -*cyclo*octene-carboxylic acids, which are inactive, is presumed to be in a favourable equatorial conformation owing to hydrogen crowding of the ring and restriction of rotation of the group as suggested by Huisgen[4] concerning the conformation of 1,2-benzo-1-*cyclo*heptenyl- and -1-*cyclo*octenyl-3-methyltosylates.

Thus, the grade of activity of the two series of compounds may be explained by the steric circumstances of the molecule, as in the hydrogenated 1-naphthoic acids[5].

On the other hand, since the hydrophil–lipophil balance may not differ significantly between the corresponding members of the two series possessing the same number of carbon atoms, the abrupt drop

Table 1. Plant-Growth Activity in the Pea Straight-growth Test*

Acids	Concentration (mol./l.) 0	5×10^{-7}	5×10^{-6}	5×10^{-5}	$1\cdot5\times10^{-4}$	$2\cdot5\times10^{-4}$	5×10^{-4}	$1\cdot5\times10^{-3}$
1,2-Benzo-1-*cyclo*butene-3-carboxylic (m.p. 74·5°–75·5°)	100	100	108	142	144	143	144	127
1-Indancarboxylic (m.p. 57·5°–58·5°)	100	—	99	138	140‡	139	134	127§
1,2,3,4-Tetrahydro-1-naphthoic (m.p. 83°–84°)	100	100	120	143	141	140	137	112
1,2-Benzo-1-*cyclo*heptene-3-carboxylic (m.p. 117°–118°)	100	—	100	99	100‡	100	103	104§
1,2-Benzo-1-*cyclo*octene-3-carboxylic (m.p. 109°–110°)	100	—	98	96	95	91	90	—
α-Methylphenylacetic (b.p. 160°–163°/25 mm.)	100	100†	108	141	137	139	129	115
α-Ethylphenylacetic (m.p. 40°–41°)	100	100	109	137	137	135	128	114
α-*n*-Propylphenylacetic (m.p. 51°–52°)	100	—	102	119	131	131	118	106
α-*n*-Butylphenylacetic (b.p. 149°–151°/4 mm.)	100	—	99	97	96	94	94	—
α-*n*-Amylphenylacetic (m.p. 35°–36°)	100	—	101	99	93	92	91	—

* The results observed for α-alkylphenylacetic acids agree with those of Veldstra, who used the pea curvature tests (ref. 2).
† At $2\cdot5\times10^{-6}$ mol./l.
‡ At 1×10^{-4} mol./l.
§ At 1×10^{-3} mol./l.

of activity in each series from the C_{11}-acid to the C_{12}-acid may be understood by an increase in lipophilic nature beyond optimum, like toxicity in the homologous series of plasmoquin, bactericidal activity in *n*-alkanols, etc.[6]. Consequently, it cannot be decided whether this abrupt change is attributable to molecular form or hydrophil–lipophil-balance, though the plant-growth activity may be conditioned by the above two factors as suggested by Veldstra[7]. From this point of view it is hoped to obtain information about the activity of homologues of 1,4-dihydro-1-naphthoic acid, which are considered to have a higher chance of having their carboxyl group in the axial positions, owing to the smaller amount of hydrogen crowding.

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Evidence for Multiple Nature of Cellulase of *Myrothecium verrucaria*

Conflicting views are held on the multiple nature of cellulase of *M. verrucaria*. Paper and column chromatography[1,2], zone electrophoresis[3] and electrophoresis-convection[4] of crude preparations of the cellulase indicated the multiple nature of this enzyme system. On the other hand, by fractionating the cellulase by precipitation techniques, Whitaker[5] obtained a preparation homogeneous in the ultracentrifuge and on electrophoresis, which exhibited all the activities tested of the crude enzyme concentrated in about the same ratios. He maintains that 1,4-β-linked oligo- and poly-glucosides are hydrolysed by one enzyme[6] which possibly forms stable complexes with metabolic products, thus giving rise to a number of components with cellulolytic activity[6,7].

In our investigations ion-exchange chromatography was used as a means to purify and/or fractionate the cellulase of *M. verrucaria*. 'Amberlite *IRC*–50' (120–200 mesh) was equilibrated with the buffer from which the cellulase was to be adsorbed. The cellulase was applied at the top of the column in a small volume of the buffer, and afterwards washed down with the same buffer. The column was operated at a speed of about 15 ml./cm.^{2} per hr. Elution was performed with buffers of increasing *p*H and/or molarity. The effluent was collected in fractions which were analysed for cellulolytic activity towards sodium carboxymethylcellulose.

Table 1

	Relative hydrolytic activity per mgm. nitrogen of the various fractions of cellulose towards:					
	o-Nitrophenyl-β-D-glucoside	Cellohexaose	Soluble cellodextrin	Sodium carboxymethylcellulose	Swollen cellulose	Cellulose
Original enzyme	100	100	100	100	100	100
Fraction *A*	5	60	50	40	30	15
B	0	110	100	60	45	0
C	5	220	210	170	120	90
D	25	100	80	120	120	100
E	40	180	110	160	140	130
F	260	160	100	140	100	130

The cellulase used was a culture filtrate of *M verrucaria* grown on cellulose powder, which was concentrated about tenfold in a Bartholomew evaporator, afterwards dialysed against running tap-water (using collodion membranes), ultrafiltered, dialysed against distilled water and freeze-dried.

Cellulolytic activity towards sodium carboxymethylcellulose was measured by incubating 5 ml. of a 0·7 per cent solution of the substance (degree of substitution, 0·8) in buffer (*p*H 5·6) with 1 ml. enzyme solution for 1 hr. at 30° C. Reducing groups formed in 1 ml. of the incubation mixture were measured and expressed as 'mgm. glucose'. The amount of enzyme producing 0·4 'mgm. glucose' under these conditions was denoted 1 cellulase unit. The enzyme solutions tested were always diluted so as to contain 1 cellulase unit or less per ml.

The results of some preliminary experiments were as follows. No adsorption of cellulase was found from a buffer solution of *p*H 6·0 (0·048 *M*). Almost all the cellulase was adsorbed from a solution buffered at *p*H 4·2 (0·048 *M*), but the cellulase could be eluted only very incompletely.

At *p*H 5·2 (0·048 *M*) 70–75 per cent of the cellulase was adsorbed. Elution with buffers of higher *p*H and/or molarity gave recoveries of 80–90 per cent of the activity towards sodium carboxy-methylcellulose. On rechromatography under identical conditions of the cellulase which was not adsorbed, 90 per cent passed through the column.

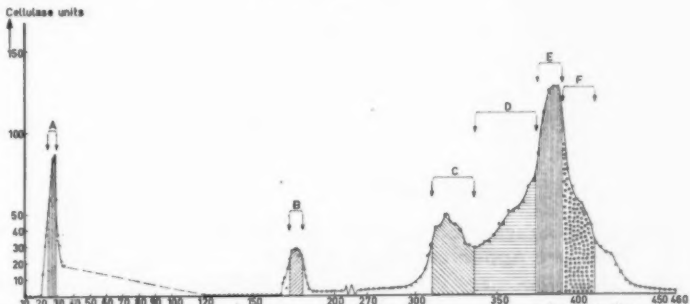

Fig. 1. Fractionation of 500 mgm. cellulase over a column of 'Amberlite *IRC*-50' (diam. 15 mm., length 73 mm.) equilibrated with phthalate buffer *p*H 5·2 (0·01 *M*) and eluted with the same buffer of increasing molarity. Fractions of 5 ml. were collected and the activity of each fraction towards sodium carboxy-methylcellulose was measured and expressed in cellulase units (see text)

The best results have been obtained so far with a column buffered with phthalate buffer *p*H 5·2 (0·01 *M*), eluting with the same buffer of increasing molarity. Fig. 1 shows the fractionation obtained. Peak *A* contains the cellulase not adsorbed. The fractions were combined as denoted in Fig. 1. Recovery of total activity applied to the column: for peak *A*, 6 per cent; *B*, 3·2 per cent; *C*, 10·1 per cent; *D*, 17·3 per cent; *E*, 17·7 per cent; *F*, 11·2 per cent; other fractions, 11·2 per cent. Total recovery 77 per cent.

Of the combined fractions *A*–*F* and of the original enzyme, dilutions were made containing the same activity towards sodium carboxy-methylcellulose (10 cellulose units/ml.) and of these the hydrolytic activity was measured towards the substrates: *o*-nitrophenyl-β-D-glucoside (niphegluc), cellohexaose, soluble cellodextrins, phosphoric acid swollen cellulose and α-cellulose. Protein concentrations were determined by the micro-Kjeldahl method. Table 1 shows the relative activities per mgm. nitrogen of the fractions when the activity of the original enzyme towards the substrate is given the value 100.

These results show that the fractions obtained by ion-exchange chromatography differ in the ratios of their activities towards a series of substrates of varying degree of polymerization. Especially fraction *B* is of great interest, as it does not hydrolyse niphegluc (nor cellobiose) nor α-cellulose, whereas its activity towards substrates of medium size (cellohexaose, cellodextrins) is as high as that of the original enzyme. Fraction *C* shows an increase in activity towards medium size substrates relative to the original enzyme. Most of the activity towards *o*-nitrophenyl-β-D-glucoside (and cellobiose) is present in fraction *F*. In our opinion these results strongly indicate the multiple nature of cellulase of *M. verrucaria*. Better resolution of the enzymes should be possible by refining the chromatography technique.

More details of this work, and further progress, will be published shortly.

We are indebted to Dr. P. Kooiman of the Laboratory of Technical Botany, Delft, who kindly provided the samples of cellohexaose and soluble cellodextrins.

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PHYSIOLOGY

Effects of Ataractic Drugs on the Blood-Pressure and Heart-Rate of Dogs

The assessment of the hæmodynamic effects of ataractic drugs, such as the phenothiazine derivatives, has, in the past, always been carried out in anæsthetized or curarized animals[1–5]. Since the action of the ataractic drug may modify, or be modified by, the effect of the anæsthetic or neuromuscular blocking agent, it has been difficult to assess whether the hæmodynamic changes observed have been due to the ataractic drug itself. It is therefore of interest to record a method of assessment which has been used for studying the effects produced by several phenothiazine derivatives in normal conscious dogs, and which has been shown to produce results which do not entirely agree with those obtained in anæsthetized animals.

Three healthy young dogs weighing 15–20 kgm. were surgically prepared with exteriorized carotid arteries and trained to lie quietly on a table while experimental procedures were being carried out. Standard limb lead electrocardiograms, and the blood-pressure obtained by puncture with a needle of the exteriorized carotid artery, were recorded simultaneously on a direct-writing multichannel recorder (Cambridge Instrument Co., Ltd.). The drugs were administered as a 1 per cent solution by intravenous injection through a previously inserted Mitchell needle at a dose-rate of 1 mgm. per kgm. body-weight. Recordings were taken before, during and after injection and every 5 min. thereafter until the maximum effect was observed (usually about 20 min. from the time of injection). Control experiments in which only saline was injected were also performed.

Seven phenothiazine derivatives were administered, in turn, to each dog:

(1), 2-chloro-10-(3-dimethylaminopropyl) phenothiazine hydrochloride (chlorpromazine hydrochloride)
(2), 10-(3-dimethylamino-2-methylpropyl) phenothiazine tartrate (trimeprazine tartrate)
(3), 10-(1-methyl-3-piperidylmethyl) phenothiazine (pecazine)
(4), 10-(2-dimethylaminopropyl) phenothiazine hydrochloride (promethazine hydrochloride)
(5), 10-(3-dimethylaminopropyl) phenothiazine hydrochloride (promazine hydrochloride)
(6), 10-(3-dimethylamino-2-methylpropyl)-2-methoxyphenothiazine hydrochloride (methotrimeprazine hydrochloride)
(7), 10-(3-dimethylamino-2-methylpropyl)-2-ethylphenothiazine hydrochloride (ethyl*iso*butrazine in the United States, 6484 *R.P.*).

The results showed that all these drugs caused significant initial tachycardia (drugs 3, 5 and 7, $P < 0{\cdot}001$; drugs 1 and 2, $P < 0{\cdot}01$ and drugs 4 and 6, $P < 0{\cdot}02$), but after 20 min. the changes in heart-rate were no longer significant. All the drugs appeared to produce an immediate effect on blood pressure, but on analysis it was shown that only drugs 1 and 7 caused significant hypotension ($P < 0{\cdot}01$), while drug 3 brought about a significant rise in blood-pressure ($P < 0{\cdot}01$). After 20 min. only drugs 1 and 7 were producing marked hypotension ($P < 0{\cdot}001$), while drug 5 was causing a moderate decrease in blood-pressure ($P < 0{\cdot}02$).

These results did not appear to agree with those which have been reported in anæsthetized animals and further experiments were performed to assess the effect of pentobarbitone anæsthesia on the response of blood-pressure and heart-rate to chlorpromazine. These experiments showed that the decrease in blood pressure from the pre-anæsthetic control-level, seen after the administration of chlorpromazine, was proportional to the depth of anæsthesia at the time of injection of this drug. Furthermore, the maximal effect on blood-pressure was obtained within 20 min. in the conscious dog, but under anæsthesia the maximum effect was not apparent until at least 30 min. after the injection of the drug. Anæsthesia also greatly reduced the immediate increase in heart-rate produced by chlorpromazine.

This investigation was carried out with the financial assistance of May and Baker, Ltd. The statistical

analyses were carried out by Dr. R. C. Campbell and Dr. J. T. Abrams.

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Influence of the Ionic Strength of the Medium on the Anaphylactic Sensitization of Isolated Tissues *in vitro*

The technique of *in vitro* sensitization of living tissues has permitted us to study various factors affecting the process of anaphylactic sensitization. The influence of the time of incubation, concentration of antibody, temperature, *p*H and composition of the medium has been already reported[1–4]. In this communication we present the results concerning the dependence of the sensitization on the ionic strength of the incubating antibody solution.

The experiments were performed with the guinea pig ileum, passively sensitized *in vitro* by incubation with rabbit, anti-egg albumin antibody, according to the technique previously described[2,3]. The antibody solution was prepared not in the usual Tyrode solution but in a medium almost free of electrolytes and made isotonic by the addition of dextrose. The composition of the medium was: dextrose 50 gm., sodium bicarbonate 42 mgm., distilled water 1 litre. Its *p*H, in equilibrium with air at 37° C., was approximately 7. Prior to sensitization the strips of intestine were washed with isotonic dextrose. During the period of incubation, the ionic concentration of the medium rose to, but never exceeded, 0·002μ (expressed as sodium chloride), presumably due to release of ions from the isolated intestine. When the influence of the ionic strength (μ) was investigated, varying amounts of sodium chloride, potassium chloride, calcium chloride, magnesium sulphate or potassium bromide were added. The salt concentration has been determined by conductivity measurements. In some experiments, dextrose was replaced by fructose, lactose, saccharose or arabinose.

The degree of sensitization was evaluated by the height of the contraction of the intestine produced in normal Tyrode solution after the addition of the specific antigen, and compared with that produced by a known dose of histamine.

Pieces of guinea pig intestine from the same animal were sensitized with the same amount of antibody in normal Tyrode solution and in dextrose medium. The intensity of the anaphylactic response of the intestine has been established as a function of the time of incubation in both media. It was found that the degree of sensitization was markedly increased in the medium with low electrolytic concentration. The results are shown in Fig. 1.

Dextrose can be replaced by fructose, saccharose, lactose or arabinose with identical results. This would suggest that the increased velocity of sensitization is related to the low concentration of electrolytes in the medium. To confirm this hypothesis we have studied the influence of various electrolytes at different concentrations, and in order to compare their respective effects, we have established, in every case, the time necessary to attain the same degree of sensitization. It was found that, for a given electrolyte, this time increased with its concentration. Furthermore, when different electrolytes were compared at the same ionic strength, their effect was found sensibly the same, regardless of their chemical nature. This result has been verified with sodium chloride, potassium chloride, calcium chloride, potassium bromide

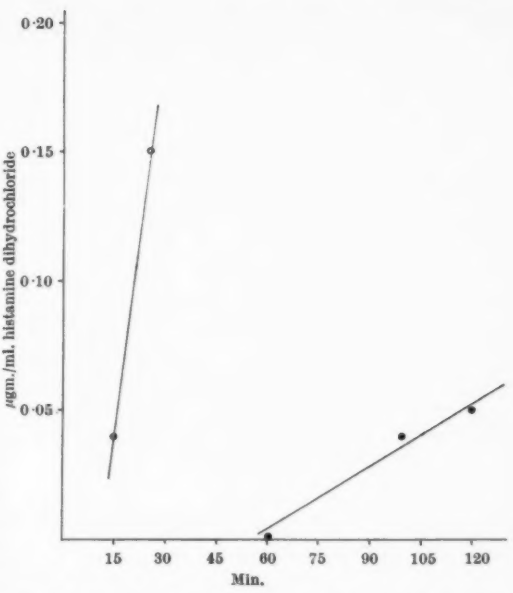

Fig. 1. Isolated guinea pig intestine sensitized passively *in vitro* at 37° C. with the same concentration of rabbit anti-egg albumin antibody (2 μgm. N/ml.). ●, sensitization in normal Tyrode solution ($\mu = 0{\cdot}15$); ○, sensitization in isotonic dextrose ($\mu = 0{\cdot}002$). Ordinate, height of the contractions expressed in histamine equivalent; abscissa, time of the incubation with the antibody

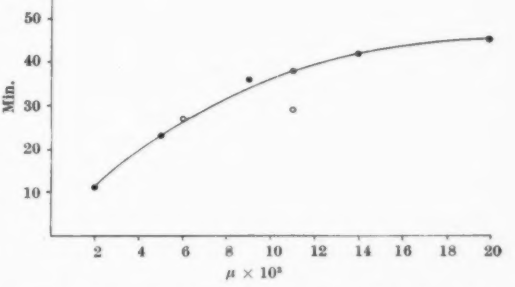

Fig. 2. Isolated guinea pig intestine sensitized *in vitro* with the same concentration of rabbit anti-egg albumin antibody (2 μgm. N/ml.) at 37° C. in media of which the ionic strength has been varied. Ordinate, time of incubation necessary to obtain an equal contraction (histamine equivalent: 0·05 μgm./ml.); abscissa, ionic strength of the incubating medium. In all experiments, the passive sensitization has been achieved in isotonic dextrose of which the ionic strength has been altered with sodium chloride (●), or magnesium sulphate (○)

and magnesium sulphate. A typical experiment is shown in Fig. 2.

The fact that the velocity is dependent on the ionic strength of the medium suggests that electrical interactions between globulin molecules or between antibody and cell play some part in the process of sensitization. Accordingly, it may be expected that modification of the *p*H would modify the rate of sensitization and the dependence of this rate on ionic strength. Preliminary experiments have shown that at low values of μ (of the order of 0·003) the sensitization process is highly dependent on the *p*H of the incubating solution.

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Use of Diodone in Renal Studies in Sheep

Diodone (3,5-di-iodo-4-pyridone-N-acetic acid), shown by Elsom *et al.*[1], Landis *et al.*[2], and Smith *et al.*[3] to be an effective agent for the estimation of renal plasma flow and tubular function, has been used extensively in physiological studies. In 1945, Smith *et al.*[4] showed that *p*-aminohippuric acid was in some ways superior to diodone for these estimations in man because the urine and plasma blanks are small, and because diodone penetrates human red blood cells whereas *p*-aminohippuric acid does not. The red cells of dogs are permeable to both reagents.

Sheep, unlike the dog and man, normally excrete considerable quantities of hippuric acid in the urine[5]. The possibility that this endogenous hippuric acid may interfere with the estimation of *p*-aminohippuric acid is not remote when sheep are employed as experimental subjects.

A study was made of the permeability of the sheep's red blood cells to diodone by administering to six adult merino ewes, as an initial intravenous priming injection, 1·5 ml. 35 per cent diodone, and following this by a continuous intravenous infusion of 0·7 per cent diodone at the rate of 4 ml./min. over a period of 2 hr. At no time during or after the injection did the animals give any indication of being adversely affected by the diodone. Blood and urine (catheter) samples, taken at 30-min. intervals, were analysed for diodone[6] and *p*-aminohippuric acid[4].

Comparison of concentrations in the whole blood and the plasma indicated the passage of little, if any, diodone into the erythrocytes (Table 1). This was substantiated by the absence of diodone in the separated, saline-washed cells from three sheep subsequent to injection.

Analyses of the blood and urine samples collected during the periods in which diodone was administered failed to detect any reaction indicative of *p*-aminohippuric acid in any of the blood samples. A reaction of the same type as that given by *p*-aminohippuric acid, however, was always detectable in the urine; the concentration of this substance, which varied considerably with animals and with samples from the same animal, indicated excretions, which ranged from 0·2 mgm. to 1·1 mgm. for a 30-min. period. This is of the same order as that found by Smith *et al.*[4] to be excreted in similar circumstances by man and the dog. Thus the blanks arising from this substance are unlikely to interfere with the employment of *p*-aminohippuric acid for the determination of renal clearance tests in sheep.

It has also been established that the red cells of sheep are impermeable to circulating diodone, and thus differ from human red cells and from those of the dog.

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Lack of Hæmatopoietic Potency of Liver of Mice fed on a Meat Diet

Recently, Adler[1] reported that mice fed on a diet composed of meat only develop an anæmia which is generally fatal but is cured by beef liver. Ilan, Kende and Guggenheim[2] have found that treatment of mice while subsisting on the meat diet with *para-*

Table 1. Showing Degree of Entry of Diodone into Erythrocytes of Sheep. Diodone Values (mgm./100 ml.) taken at 30-min. Intervals of Time

Animal No.	Plasma				Whole blood				Calculated whole blood*				Entry of diodone into erythrocytes (per cent)			
	30	60	90	120	30	60	90	120	30	60	90	120	30	60	90	120
2	1·92	1·73	1·97	1·78	1·15	1·08	1·15	1·10	1·22	1·10	1·24	1·14	—	—	—	—
81	1·78	1·48	1·56	1·56	1·14	0·97	1·01	1·01	1·18	0·97	0·99	1·00	—	—	2	1
31	0·88	1·32	1·58	1·65	0·51	0·86	0·99	1·14	0·58	0·87	1·06	1·12	—	—	—	1·9
58	1·96	1·83	1·69	1·69	1·32	1·25	1·12	1·14	1·29	1·21	1·14	1·14	2·3	3·3	—	—
60	2·06	2·02	1·96	1·40	1·29	1·29	1·24	0·93	1·29	1·29	1·26	0·92	—	—	—	1·1
9	1·86	1·70	1·70	1·57	1·16	0·95	0·95	0·99	1·15	1·08	1 08	1·01	0·9	—	—	—

* Calculated whole blood = Plasma value × hæmatocrit value (for plasma).

aminobenzoic acid, folic acid, vitamin B_{12}, liver extract, pyridoxine, iron, vitamin A, antibiotics (*per os*) or a combination of some of these substances neither prevents the anæmia nor the mortality nor does it improve growth. Some of these substances have, however, a slight effect in preventing anæmia. It could furthermore be shown that livers of mice suffering from 'meat anæmia' contained much more vitamin B_{12} than those of non-anæmic controls. It has been assumed that an essential hæmatopoietic factor, which is not vitamin B_{12}, folic acid or any other known hæmatopoietic factor, is absent from beef muscle; another possibility is the presence in beef muscle of an inhibitor of hæmatopoiesis.

We wish to report some further experiments conducted with the purpose of studying the effect of an addition of liver to the diet. 42 mice rendered anæmic by feeding the meat diet for 6 weeks were divided into 4 groups. One group served as control and continued on the meat diet. The diet of the three other groups consisted of three parts beef muscle and one part liver. The source of the liver of one of these groups was beef, that of the second, normal mice, and that of the third, anæmic mice. This dietary treatment lasted for 3 weeks. Table 1 presents figures for mortality, change in weight, erythrocytes and hæmoglobin. It shows that replacement of one of four parts of beef muscle by beef liver cured the anæmia completely and induced a considerable increase in body-weight. Liver of normal mice had a definite but smaller effect, and liver of anæmic mice resulted in no improvement.

Table 1. MORTALITY, WEIGHT CHANGES, RED BLOOD CELL COUNTS AND HÆMOGLOBIN OF ANÆMIC MICE FED ON LIVER INCORPORATED INTO A MEAT DIET

Source of liver	Period of treatment	Mortality	Weight (gm.)	Erythrocytes (10^6/cu. mm.)	Hæmoglobin (gm./100 ml.)
None, control (10)	Pre-treatment	—	14·6±1·4	3·04±0·13	6·6±0·8
	3 weeks	7/13	13·4±1·7	2·84±0·54	3·5±0·8
Beef liver (6)	Pre-treatment	—	17·0±1·8	2·26±0·10	5·8±0·9
	3 weeks	2/6	21·0±0·9	6·82±0·23	15·8±0·5
Normal mouse liver (13)	Pre-treatment	—	18·4±0·6	3·14±0·40	5·3±0·54
	1 week	0/13	17·5±0·6	3·65±0·49	7·2±1·22
	2 weeks	1/13	19·5±0·7	4·42±0·63	8·1±1·04
	3 weeks	4/13	20·1±0·8	5·39±0·68	9·8±1·17
Anæmic mouse liver (13)	Pre-treatment	—	16·2±1·0	2·68±0·37	5·6±0·80
	1 week	2/13	16·0±1·1	2·36±0·23	4·4±0·58
	2 weeks	9/13	15·8±1·6	1·65±0·23	2·8±0·18
	3 weeks	11/13	15·8	1·42	2·3

These results are consistent with the theory that a hæmatopoietic factor is present in beef liver in higher concentration than in normal mice liver and is absent from liver of mice which had subsisted on beef muscle. The existence in liver of an anti-anæmic factor(s) other than vitamin B_{12}, folic acid and folinic acid has repeatedly been claimed[3].

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Effect of Anti-Diuretic Hormone and Calcium on the Equivalent Pore Radius of Kidney Slices from *Necturus*

THE passive permeability of kidney slices may be expressed in terms of the equivalent pore radius. The present work is concerned with measurement of this parameter for kidney slices from the amphibian, *Necturus maculosus*, both under normal conditions, and as affected by the action of anti-diuretic hormone and calcium. Measurements have been made by the method of D. A. Goldstein and A. K. Solomon (private communication), which depends on the determination of the external concentration of permeant solute required to prevent water movement into or out of the cell at the instant of immersion in the medium. When the initial rate of change in volume of the cell is zero, the cell is in transient osmotic equilibrium with its environment. As Staverman[1] has pointed out, the concentrations required to produce transient osmotic equilibrium are higher for permeant solutes than for impermeant ones. Goldstein and Solomon have shown how these zero-time isotonic concentrations may be related to the equivalent pore radius.

Slices (0·2 mm. thick, 100–200 mgm. wet weight) from the kidneys of *Necturus* were weighed and immersed in a number of test solutions. Changes in the weight of the slice, which served as an index of cell volume change, were measured for 30 min. At 3–4-min. intervals, each slice was taken out of the solution, blotted, weighed and returned to the solution. Since the rate of change of weight was essentially constant during the first 5–10 min., an average during that period was determined and recorded as the per cent weight change per minute. All the bathing solutions contained sodium bicarbonate, 21 mM; dipotassium hydrogen phosphate, 0·6 mM; sodium dihydrogen phosphate, 2·4 mM; potassium chloride, 13 mM; magnesium sulphate, 1·2 mM; sodium sulphate, 0·6 mM; calcium chloride, 1 mM and glucose 5·5 mM, making a total of 78 mOsm./l. In addition, one of the following non-lipid-soluble non-electrolytes was added: raffinose, sucrose, mannitol, erythritol, glycerol or urea. The final total concentration ranged from 125 to 300 mOsm./l. *p*H was kept at 7·3 ± 0·1 by bubbling with 95 per cent oxygen and 5 per cent carbon dioxide. The experiments were performed at 18–20° C.

The zero-time rate of change of slice weight, $(dw/dt)_0$, is obtained by extrapolation. It is shown in Fig. 1 as a function of the concentration of probing molecule in the medium. Each point is the average of results obtained from 8–12 slices from 4–6 kidneys. It may be seen that $(dw/dt)_0$ for sucrose is zero at a concentration of 83 mM, as is also the case for raffinose and mannitol at the same concentration.

Table 1. C_{iso} AND σ FOR NON-LIPID-SOLUBLE NON-ELECTROLYTES IN KIDNEY SLICES

	No 'Pitressin'		'Pitressin'		Molecular radius* Å.
	C_{iso} (mM)	σ	C_{iso} (mM)	σ	
Urea	160 ± 10†	0·52	160	0·52	2·3
Glycerol	108 ± 4	0·77	128	0·65	2·9
Erythritol	93 ± 3	0·89	108	0·77	3·2
Mannitol	83 ± 4	1·0			4·0
Sucrose	83 ± 3	1·0	83	1·0	4·5
Raffinose	83 ± 3	1·0			6·0

* Molecular radii were determined on Stuart and Briegleb models, except raffinose, taken from viscosity measurements (ref. 8).
† Errors are standard errors of the mean.

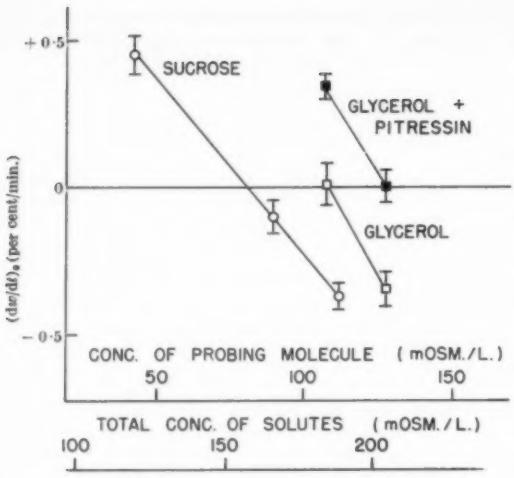

Fig. 1. Initial change in weight plotted as a function of the concentration of solute in the bathing solution. C_{iso} for sucrose may be seen to be 83 mOsm./l. compared with 108 for glycerol

Since we found the volume of distribution of mannitol to be the same as that of inulin in these kidney slices, it appears that all three solutes are confined to the extracellular phase during the course of our experiments. Consequently, the 83 m*M* concentration has been taken as the base line for comparison with experiments utilizing permeant solute. The zero time isotonic solution concentrations, C_{iso}, are given in Table 1. The determination of C_{iso} is equivalent to the determination of Staverman's reflexion coefficient, σ, also shown in Table 1. σ is given by the ratio (osmotic pressure due to permeant solute)/(osmotic pressure that would arise if solute were impermeant).

Goldstein and Solomon (private communication) have constructed a family of theoretical curves which

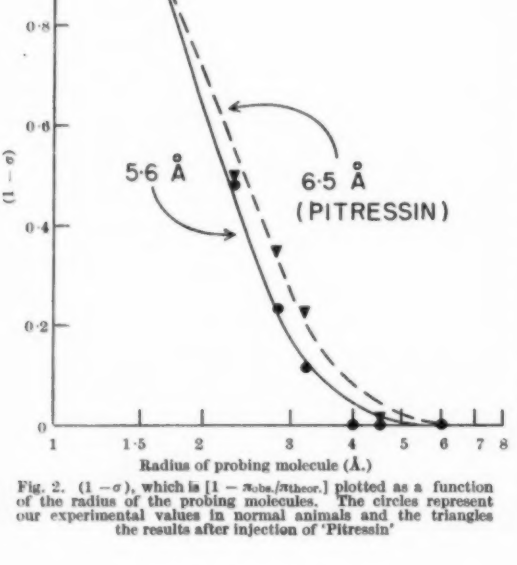

Fig. 2. $(1-\sigma)$, which is $[1 - \pi_{obs.}/\pi_{theor.}]$ plotted as a function of the radius of the probing molecules. The circles represent our experimental values in normal animals and the triangles the results after injection of 'Pitressin'

relate $(1 - \sigma)$ to the equivalent pore radius in the membrane and to the radius of the probing molecule. They applied these curves to their measurements of σ and obtained a figure of 4·2 Å., for the equivalent pore radius in human red cells, in agreement with the value of 3·5 Å. obtained by an independent method[2]; this agreement lends support to the use of σ to determine equivalent pore radii for such small pores. The curves given by Goldstein and Solomon (private communication) may also be applied to the data in Table 1. As shown in Fig. 2, an equivalent pore radius of 5·6 Å. fits our results. Although the slices are composed predominantly of proximal tubule cells, they contain also other cell types. Thus the figure of 5·6 Å. represents an average over all the types of cells that are encountered within the slice.

The effect of anti-diuretic hormone ('Pitressin', Parke Davis and Co.) was examined in a group of experiments in which 10 pressor units of 'Pitressin' per animal were injected intraperitoneally 2½–3½ hr. before the kidneys were taken out. As shown in Table 1, no change in C_{iso} for sucrose was observed; however, C_{iso} for erythritol and glycerol decreased significantly ($P = 0{\cdot}01$). When these results are plotted in Fig. 2, it is seen that the equivalent pore radius is increased to 6·5 Å. Results compatible with this observation have previously been described by Ussing for toad skin[3], and by Sawyer and Hays *et al.*[5] for amphibian bladder.

Table 2. Inhibition of 'Pitressin' Action by Calcium in *Necturus* Kidney Slices

Calcium (m*M*)	Initial rate of weight change in isotonic glycerol* 1	5 (per cent/min.)	10
No 'Pitressin'	0·01 ± 0·07	0·00 ± 0·04	0·01 ± 0·06
'Pitressin'	0·34 ± 0·04	0·17 ± 0·04	−0·03 ± 0·06
'Pitressin' induced effect	0·33 ± 0·08	0·17 ± 0·06	−0·04 ± 0·08
P of induced effect	< 0·01	0·02	> 0·6

* The solutions were maintained isotonic by substitution of calcium chloride for sodium bicarbonate. The glycerol concentration was 108 mOsm./l. The osmolarities of the solutions were checked by freezing-point depression.

The absence of calcium is known to cause cell swelling, as shown by Fukuda[6] in *Anthocidaris* eggs, and by Bozler[7] in muscle. Consequently, it seemed desirable to examine the influence of calcium on our preparation. An increase of calcium from the normal value of 1 m*M* up to 10 m*M* had no detectable effect on $(dw/dt)_0$ when the cells were immersed in sucrose solutions. On the other hand, removal of calcium from the medium produced a sharp increase in weight, the tissue becoming permeable to sucrose (molecular radius 4·5 Å.), though remaining impermeable to raffinose (molecular radius 6·0 Å.). It may be inferred that the calcium content of the membrane exercises a delicate control over the equivalent pore radius. Consequently, a set of experiments was carried out to see whether excess calcium might inhibit the action of anti-diuretic hormone. The results shown in Table 2 indicate that this is indeed the case. When the slices are immersed in isotonic glycerol, 5 m*M* calcium reduces the effect of 'Pitressin' to half and 10 m*M* calcium suppresses it. A similar effect has been observed when the slices are immersed in isotonic erythritol, 10 m*M* calcium completely inhibiting the 'Pitressin' effect. These results suggest that the action of anti-diuretic hormone on membrane permeability may be due to an interaction with membrane calcium.

This work was supported in part by grants from the Life Insurance Medical Research Fund and the National Science Foundation.

Note added in proof. Since this communication was submitted, we have seen a paper by P. J. Bentley (*J. Endocrin.*, **18**, 327; 1959), who observed a similar interaction between calcium and 'Pitressin' on toad bladder.

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HÆMATOLOGY

MNSs Gene Frequencies in English Blood Donors

Estimates of the MNSs gene frequencies in the English population have been based hitherto on the results of tests with anti-M, -N and -S only[1,2]. Recently, a supply of the very rare anti-s was made available through the kindness of Dr. J. O'Riordan of Dublin[3], and a random series of 1,000 Sutton blood donors was tested with all four antisera. The samples were also tested with anti-Mi[a], -Vw and -M[g]. All were negative with the last two antisera, but one donor was found to be *Ms/Ns*, Mi(a +) Vw−, and a study of her family showed *Mi*[a] to be travelling with *Ns*. The results of the major grouping tests are given in Table 1.

Table 1. Samples from 1,000 Unselected Sutton Blood Donors tested with Anti-M, -N, -S and -s

Reactions with anti				Number
-M	-N	-S	-s	
+	−	+	−	57
+	−	+	+	140
+	−	−	+	101
+	+	+	−	39
+	+	+	+	224
+	+	−	+	226
−	+	+	−	3
−	+	+	+	54
−	+	−	+	156
			Total	1,000

Table 2. Percentage Frequencies of the MNSs Genes in English People

	Sutton series, 1959 Calculations based on the use of Anti-M,-N,-S-s	Anti-M,-N,-S	Race and Sanger (ref. 1) 1950	Ikin *et al.* (ref. 2) 1952
MS	23·11 ± 1·022	23·58 ± 1·116	24·72	24·02
Ms	30·90 ± 1·102	30·67 ± 1·185	28·31	29·71
NS	7·09 ± 0·678	6·89 ± 0·807	8·02	5·64
Ns	38·90 ± 1·155	38·86 ± 1·233	38·95	40·63
No. tested	1,000		1,419	1,166

Gene frequencies have been calculated by the method of maximum likelihood estimation, and are presented in Table 2. They have also been calculated from the same data, assuming the use of anti-M, -N and -S only; these results are included in Table 2 for comparison along with those of Race and Sanger[1], and of Ikin *et al.*[2], whose tests were in fact made with the three sera only. The calculated gene frequencies, when recombined, give expected figures for the 9 phenotype classes which fit very closely with those observed ($\chi^2 = 3{\cdot}64945$ for 4 d.f.)

I am indebted to Dr. J. H. Edwards of the Medical Research Council Population Genetics Research Unit, Oxford, for the statistical treatment. He has estimated from these results that tests on 800 samples with all four antisera may be expected, on average, to yield as much information as tests on 1,000 samples with the three antisera more readily available.

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Chemical Constitution of the Red Blood Cell Membrane

Some authors[1-6] claim that the red blood cell membrane is a continuous lipoid. Others[7-10] do not accept this. They assert that the membrane consists of a protein skeleton with lipoid molecules in the hollows of the protein skeleton.

The work reported here aims at settling this with the help of the Sanger dinitrofluorobenzoic acid method for amino end-group determination[11].

Red cells freed from native protein and its fraction were used. Fractionation was as follows: to 10 ml. red cell suspension 20 ml. physiological salt solution and 10 ml. ether were added. This was shaken for 5 min. and then centrifuged. Three phases resulted: the central layer (the stroma) was solid; the upper ether phase contained lipoid; the lower solution contained hæmoglobin. The extraction was repeated five times. We combined the fraction obtained in the course of the extractions. The stroma and the watery solution from ether were dried and the ether solution evaporated. Thus we obtained ether-soluble lipoid- and hæmoglobin-free stroma, ether hæmolysate and such lipoid fraction as contained any ether-soluble fat-like matter.

Since the alcoholic solution of dinitrofluorobenzoic acid causes hæmolysis on native red cells, the method had to be modified. The reactions were performed with native red cells and aqueous solution, with physiological salt of dinitrofluorobenzoic acid at *p*H 8·0 (0·15 *M* HCO_3–CO_3), at 37° C. by slight stirring for 60 min. Hydrolysis of the products was effected with 6 *N* hydrochloric acid at 105° C. (6 hr.). Analysis of purified dinitrophenyl derivatives was performed with paper chromatography[12].

The chromatogram of native red cell and of its three fractions is illustrated in Fig. 1.

Each substance was identified by its mobility and by the application of model compounds.

On the surface of the native red cell[9] two amino-acids, arginine and serine amino end-groups, are to be found. Serine can be derived both from stromatin-proteins and from cephalin; consequently its presence is not conclusive. The arginine, however, is exclusively of protein origin, therefore the stroma-proteins are not likely to be encircled by coherent lipoid membrane. This fact is confirmed by Block and Bollding[13], who

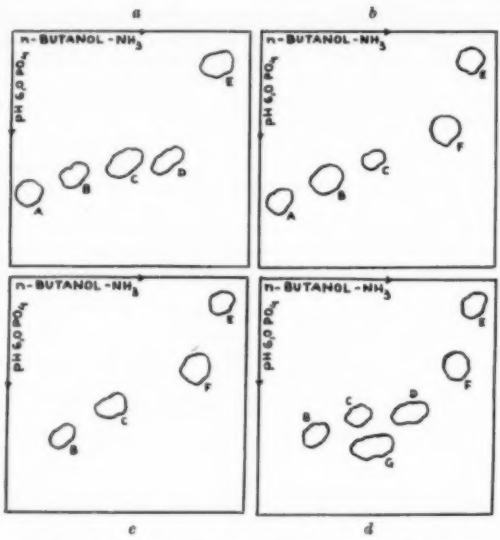

Fig. 1. *a*, Native red cells. *A*, DNP-arginine; *B*, DNP-serine; *C*, unknown amine; *D*, unknown amine; *E*, DNP-NH_2. *b*, Lipid-free stroma; *F*, DNP-colamin. *c*, Lipid extraction. *d*, Hæmolysate; *G*, DNP-valine

find that the arginine content of stroma-protein is very high. After the removal of a greater part of lipoids by ether from the stroma obtained further end-groups originating from amino-acid could not be observed. The fact that serine is to be found in the stroma, too, confirms its protein or cephalin origin, which in greater part is attached to protein, although it probably occurs in the form of lipoprotein. The latter is confirmed also by the fact that colamin can be found in the stroma in great quantities, which can be isolated in the form of dinitrophenyl-colamin. In the lipoid fraction removed from the red cell by ether, serine and an unknown amino compound were found (Fig. 1*c*).

The possibility arose that the dinitrofluorobenzoic acid did not react on the surface of the red cell only but also in its interior. Our experiments have shown that this is not the case, because in the hæmolysate of typical hæmoglobin origin[11] 2 mol. valine end-group appeared; in the case of the native red cell this product could not be found.

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PATHOLOGY

High-Energy Bound Phosphate and Diabetes Mellitus

The problem of whether oxidative phosphorylation is impaired in diabetes mellitus or not remains obscure. Cutolo and Siliprandi[1] and Sacks[2] demonstrated a decrease in high-energy bound phosphate content in alloxan-diabetic rat liver. Vester and Stadie[3] found diminished oxidative phosphorylation in depancreatized cat liver mitochondria but not in alloxan-diabetic rat liver mitochondria. Parks *et al.*[4] also failed to find an abnormality in alloxan-diabetic rat liver mitochondria. Goranson and Erulkar[5] found uncoupling of oxidative phosphorylation in the heart muscle of alloxan-diabetic rats, but Peterson and his collaborators[6] reported that the high-energy bound phosphate content of skeletal muscle was in the normal range.

In order to see whether the formation of high-energy bound phosphate is disturbed in diabetic patients, we determined the blood high-energy bound phosphate in 65 diabetic patients and 63 non-diabetic control subjects. The blood high-energy bound phosphate content in the overnight fasting state was determined as 10 min. hydrolysed phosphorus by the method described elsewhere[7]. The values obtained by the method showed a fair agreement with those obtained by the method of Wolff and Paysant[8]. The diabetic patients studied were non-ketotic. In patients treated with insulin, blood specimens were obtained after the omission of regular insulin for 24 hr.

The mean 10-min. phosphorus in blood level was $3{\cdot}4 \pm 0{\cdot}09$ mgm./100 ml. for the diabetic group and $3{\cdot}5 \pm 0{\cdot}11$ mgm./100 ml. for the control group and there was no significant difference statistically ($P > 0{\cdot}10$). The mean 10 min. phosphorus content of blood cells was $7{\cdot}7 \pm 0{\cdot}17\gamma \times 10^{-9}$ per red cell for the diabetic group and $7{\cdot}8 \pm 0{\cdot}07\gamma \times 10^{-9}$ per red cell for the non-diabetic group. Therefore, the fasting blood high-energy bound phosphate content of diabetic patients was not different from that of non-diabetic control subjects.

Our previous work showed, however, that the injection of adrenaline (0·5 mgm.)[7] or cortisone (25 mgm.)[9] depressed the level of blood 10 min. phosphorus to a greater extent in diabetic patients than in non-diabetic subjects. The increase in blood 10 min. phosphorus produced by the subcutaneous injection of insulin (5 units)[9], by the oral administration of 25 gm. of butter[10] or by the oral administration of 1 gm. of carbutamide[11] was significantly less in diabetic patients than in non-diabetics. No significant difference between diabetic patients and non-diabetic subjects was found in the changes of blood 10 min. phosphorus-level after the oral ingestion of 50 gm. of glucose[12], after the intramuscular injection of glucuronic acid (1,000 mgm.)[13] or after the oral administration of phenethylbiguanide (100 mgm.)[11]. Similar results were obtained in the study on alloxan-diabetic rabbit liver 7 min. phosphorus, that is, there was no significant difference in the liver 7 min. phosphorus in the post-absorptive state between diabetic rabbits and normal rabbits, but the change after adrenaline administration (0·01 mgm./kgm.) was significantly greater and the change after insulin (1·5 units/kgm.) was significantly less in the diabetic rabbit groups than in the normal group[14].

We feel that diabetic patients have a slight disturbance in high-energy bound phosphate formation

which becomes manifest after the administration of adrenaline, cortisone, insulin, etc.

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Recovery of Leukæmogenic Agent from Non-Leukæmic Tissues of Thymectomized Mice

It has been shown by Gross and others[1] that filtrates of leukæmic tissues are leukæmogenic following injection into new-born mice of a low-leukæmic strain, and that total thymectomy prevents the development of lymphocytic leukæmia in inoculated mice[2]. On the other hand, normal thymuses grafted to inoculated thymectomized hosts as late as 6 months after thymectomy develop lymphocytic neoplasms[3]. It follows, therefore, that a leukæmogenic agent might be recoverable from the tissues of inoculated mice up to 6 months or more after thymectomy.

Six-months old *C3Hf/Gs* mice, which had received an intraperitoneal injection of passage *A* filtrate (a potent leukæmogenic filtrate) at or soon after birth and had been thymectomized at 3–4 weeks of age, were killed, and extracts were prepared from their spleens or brains. Miroscopic examination of these organs did not reveal any leukæmic cells or any other abnormality. Some of the spleen cells were transplanted to normal adult *C3Hf/Gs* mice, but these remained free from leukæmia during a total period of observation of 8–9 months. The spleen and brain extracts were inoculated intraperitoneally into new-born *C3Hf/Gs* mice, which then received no further treatment. As can be seen in Table 1, 60–80 per cent of the mice in the experimental group developed leukæmia as early as 3 months after birth. No leukæmia developed in 13 control mice inoculated with brain and spleen extracts prepared from untreated 6-month-old *C3Hf/Gs* mice.

Extracts from non-leukæmic tissues of inoculated thymectomized hosts were passaged through successive generations of thymectomized mice and finally into non-thymectomized mice. Leukæmias are already appearing in the group with intact thymuses, suggesting that: (1) multiplication of the leukæmic agent has taken place each time during transfer through thymectomized hosts; (2) the thymus is not a necessary site for replication of the agent since in the last two generations of thymectomized hosts the material was injected after thymectomy. These results will be published in full detail when the results are complete.

I wish to thank Profs. A. Haddow and P. C. Koller for their interest. The investigations have been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council, the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

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Effect of a Primary Tuberculous Infection on the Resistance of Male and Female Mice to Ectromelia

A tuberculous infection or vaccination with *BCG* has been shown to raise the resistance of mice and guinea pigs not only to a challenge with mycobacteria but also to secondary infections caused by other species of bacteria. This latter, non-specific type of immunity has been demonstrated against infection with *Brucella abortus*[1], *Bacillus anthracis* and *Brucella suis*[2], *Staphylococcus aureus*[3], *Pasteurella pestis*[3] and *Salmonella enteritidis*[4]. The present work was undertaken to see whether a primary tuberculous infection also increased the resistance of mice to virus infections, using in the first instance ectromelia (mouse pox) as the test virus.

Male and female mice, strain *VS*, aged 7–9 weeks (weight 18–20 gm.) were each divided into two groups.

Table 1. The Development of Lymphocytic Leukæmia in Mice inoculated with Extracts of Non-leukæmic Tissues of Thymectomized Passage *A*-inoculated Mice

Donor material		Recipient		Mice with lymphocytic leukæmia		
		Number	Age at injection	Number	Age in months	Per cent
Passage *A*-inoculated thymectomized 6-months-old *C3Hf/Gs* mice	Brain extract	16	1 day	10	3–6 (av. 4·1)	63
	Spleen extract	14	1 day	11	3–5 (av. 3·8)	80
	Spleen cells	8	2–3 months	0	—	0
Untreated 6-month-old *C3Hf/Gs* mice	Brain extract	6	1 day	0	—	0
	Spleen extract	7	1 day	0	—	0

Total period of observation 8–12 months.

Table 1

Experiment No.	Tuberculous mice				Normal mice			
	Male		Female		Male		Female	
	Survivors* No. per group	Percentage of survivors	Survivors* No. per group	Percentage of survivors	Survivors* No. per group	Percentage of survivors	Survivors* No. per group	Percentage of survivors
1	8/20	40	11/19(1)†	58	4/30	13	5/10	50
2	11/30	37	12/28(2)†	43	5/30	17	15/29(1)†	52
3	13/56(4)†	23	9/56(4)†	16	2/59(1)†	3	17/55(5)†	31
Total	32/106	30	32/103	31	11/119	9	37/94	39

Mice inoculated intraperitoneally with ectromelia (experiments 1 and 2, 10 *LD*50; experiment 3, 30 *LD*50) 3 weeks after infection with *Myco. tuberculosis*.
* Seventeen days after infection with ectromelia.
† Bracketed figures indicate number of mice which died without typical ectromelia or with severe tuberculosis and were excluded from group.
Probability that difference between survivor ratios for tuberculous and normal males occurred by chance $< 0{\cdot}001$ ($\chi^2 = 14{\cdot}6$).
Probability that difference between survivor ratios for normal males and normal females occurred by chance $< 0{\cdot}001$ ($\chi^2 = 25{\cdot}6$).
Probability that difference between survivor ratios for tuberculous males and normal females occurred by chance $> 0{\cdot}2$ ($\chi^2 = 1{\cdot}47$).

One group of male and one of female mice were inoculated intravenously with 0·0065 mgm. *Mycobacterium tuberculosis* (virulent human strain, *H37Rv*) in order to produce a chronic stable type of pulmonary tuberculosis[4]. One group of each sex was left uninfected. Three weeks later, when the infected mice showed small tuberculous lesions in the lungs, all groups were inoculated intraperitoneally with a suspension of ectromelia. In the first two experiments the virus suspension contained approximately 10 *LD*50 per inoculated dose and in the third experiment 30 *LD*50 (determined with weanling mice aged 3–4 weeks). Subsequent deaths were recorded daily and the cause of death confirmed at autopsy. The few mice which died without typical lesions of ectromelia or with lesions of tuberculosis sufficiently severe to account for death were excluded from an experiment. The results of three such experiments are shown in Table 1. In all the experiments the resistance to ectromelia of the normal male mice, as indicated by the proportion of survivors, was consistently less than that of the male mice with tuberculosis. The resistance to ectromelia of normal female mice, initially higher than that of normal male mice, was not significantly influenced by a primary tuberculous infection and was approximately equal to that of the male mice with tuberculosis. The lower resistance of the normal males compared with the females may not apply to mice of all strains and ages. We have some evidence that the resistance of normal weanling mice (aged 3–4 weeks) of strain *VS* is about the same in males and females.

Under the conditions of these experiments, tuberculosis increases the resistance of adult male mice to the lethal effects of ectromelia, a typical pox virus. Although tuberculosis can increase the resistance of animals to secondary infections caused by other species of bacteria, this is the first time it has been observed with a secondary virus infection. Nevertheless it is not known whether this increased resistance to a virus infection results from the same non-specific immune processes which operate against the bacterial injections.

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RADIOBIOLOGY

Comparison of Iodine-124 and Iodine-131 in Inhibition of Goitrogenesis in the Rat

Phillips[1] has suggested that iodine-124 might prove more effective than iodine-131 in the treatment of cancer of the thyroid, largely because the greater energy of its β-rays should increase the amount of radiation administered from areas of functioning tumour to adjacent foci that do not take up iodine. We were therefore interested to compare experimentally the damaging effect on the thyroid of the two isotopes. The method used was that of Doniach and Logothetopoulos[2], who showed that radiation damage to the thyroid of rats may be revealed by their loss of ability to produce goitres in response to a 10-day course of propylthiouracil when challenged 3–4 months after a single dose of iodine-131. Marked inhibition of goitrogenesis resulted from an intraperitoneal injection of 30 μc. of iodine-131. This dose gives a mean of about 12,000 rads to the rat thyroid with the very large range of about 2,000–24,000 rads, due to uneven uptake and distribution of iodine in the thyroid and loss of β-radiation from the gland periphery. It was found that the dose of external X-irradiation to the rat's thyroid necessary to inhibit the goitrogenic response to a degree equivalent to 30 μc. of iodine-131 was only 1,000 rads[3]. It is likely that part of the greater effectiveness of external X-irradiation over iodine-131 in terms of dosage is a reflexion of the uneven uptake of iodine and consequent comparative escape from iodine-131 radiation of less-actively functioning areas of thyroid. We thought that the radiation dose of iodine-124 necessary to inhibit goitrogenesis would be less than that of iodine-131 because its more penetrating radiation might even out the dosage throughout the thyroid gland, following Phillips's[1] argument with regard to thyroid cancer.

The iodine was prepared on the Medical Research Council cyclotron at Hammersmith Hospital by bombarding antimony with α-particles of about 18 MeV. A chemical separation of the iodine produced a specimen of high specific activity (1 mc./μgm.): the iodine-124 was, however, contaminated with other radioisotopes of iodine inevitably formed in the alpha bombardment of natural antimony. These are iodine-123 (half-life 13 hr.), iodine-126 (13 days) and iodine-125 (60 days). The extent of the contamination was estimated by following a sample of the mixture in a re-entrant ionization chamber. This chamber had been calibrated with several known

isotopes, and its response was found to be linear with specific γ-ray emission if radiations below about 100 keV. are ignored. At the time of administration it is estimated that the response of the chamber to the different isotopes was in the proportions iodine-123 : iodine-124 : iodine-126 = 1·0 : 2·0 : 0·17. The proportions in terms of microcuries can be found by dividing these figures by the appropriate specific γ-ray emissions. There is some uncertainty about the specific γ-ray emission of iodine-124 (refs. 4–7); the figures for the administered quantities quoted in the table assume a value of 6·5 r./mc. hr. at 1 cm., the contribution from the 30-keV. X-rays being ignored. The estimated quantity of iodine-124 administered is inversely proportional to the figure assumed. The amount of iodine-125 present was not directly determined, but experience with previous samples indicates that it was present to the extent of only a few microcuries per 100 microcuries of iodine-124.

Table 1

Group (number of rats per group in brackets)	Thyroid weight in mgm. ± *S.D.* of mean	Percentage inhibition of goitrogenesis
Controls (11)	23·6 ± 0·8	—
Methylthiouracil (11)	48·9 ± 3·1	0
10·1 μc. iodine-131 followed by methylthiouracil (12)	44·7 ± 1·3	20
29·5 μc. iodine-131 followed by methylthiouracil (12)	34·8 ± 1·1	60
21 μc. iodine-124 followed by methylthiouracil (11)	45·3 ± 2·9	20
37 μc. iodine-124 followed by methylthiouracil (12)	40·0 ± 1·5	40
53 μc. iodine-124 followed by methylthiouracil (11)	35·0 ± 2·2	60
126 μc. iodine-124 followed by methylthiouracil (11)	27·7 ± 1·7	88
222 μc. iodine-124 followed by methylthiouracil (12)	24·7 ± 0·8	100

Thyroid weights of rats after 16 days on 0·1 per cent methylthiouracil in the drinking water, started 104 days after intraperitoneal injection of various doses of iodine-124 or iodine-131. Percentage inhibition of goitrogenesis is calculated on the assumption of 25 mgm. for weight of the untreated thyroid and 50 mgm. for the weight of methylthiouracil goitre in the absence of radiation inhibition.

105 male pen-inbred rats of the hooded Lister strain were divided into 9 groups, 11 or 12 per group. At the age of 8–9 months the rats in 7 groups were injected intraperitoneally with carrier-free iodine-131 or iodine-124 in 1 ml. water in the dose-range shown in Table 1. 104 days later the treated animals and 1 non-radiated group were put on methylthiouracil in the drinking water, 1 gm. per l., and killed after sixteen days of this goitrogenic regime together with the remaining group of non-irradiated, non-thiouracil-treated controls. There were two deaths among the 105 rats during the experiment. The average thyroid weight of the controls was 23·6 mgm., and that of the thiouracil but non-radioactive iodine-treated was 48·9 mgm. Thus the goitrogenic regime had doubled the thyroid weight. It is seen in Table 1 that 29·5 μc. iodine-131 significantly inhibited the goitrogenic response, the thyroid weight being 34·8 mgm. However, 10·1 μc. iodine-131 showed little effect, the thyroid weight being 44·7 mgm. The dose of iodine-124 equivalent to 29·5 μc. iodine-131 in inhibition of goitrogenesis was 53 μc. thyroid weight 35·0 mgm. The degrees of inhibition of goitrogenesis produced by the various doses of radioactive iodine may be expressed as percentages, as shown in Table 1 where the control thyroid weight is regarded as 25·0 mgm., and the goitrogen treated as 50 mgm. 29·5 μc. iodine-131 and 53 μc. iodine-124 each showed a 60 per cent inhibition.

To interpret this equivalence in terms of radiation dose is not easy. The dimensions of the rat thyroid are comparable with the ranges of β-particles, and calculations of radiation dose are therefore very dependent on the precise geometry of the gland and the exact ranges of the particles. If the biological half-life of iodine in the gland is taken as 100 hr., and the gland is assumed to consist of two cylinders 5 mm. long and 2 mm. in diameter, an approximate calculation shows that about 2·5 μc. of iodine-124 will give the same average radiation dose to the gland as 1 μc. of iodine-131. Our experimental result of 1·8–1 suggests that rather less than this amount of iodine-124 is required in order to produce equivalent damage. The comparison is approximate, particularly because of the uncertainty in the value of the specific γ-ray emission of iodine-124. It is, however, considered very fully in recent work by Christine M. Matthews and J. F. Fowler[8].

Our thanks are due to the cyclotron staff here for preparation of the iodine-124.

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Behaviour of Radioactive Griseofulvin in Skin

The present clinical and laboratory success of griseofulvin in the treatment of superficial fungal infection has prompted investigation into the sites of concentration of this material in an effort to clarify its effects. Preliminary work with spectrophotometry suggested that griseofulvin accumulated, at least for short periods, in the skin. Since this tissue is also the site of the primary infection, I confined my studies to the localization of the agent in that organ, using radioactive griseofulvin the presence of which could be identified by radioassay and autoradiography.

Two investigations were made using griseofulvin–chlorine-36 of specific activity 6·7 μc. per gm. In the first part 2 normal rats were fed by tube 100 mgm./kgm. of radioactive griseofulvin on three successive days. The rats were killed 4 and 7 hr. respectively after the last doses, and radioassay and spectrophotometric analyses were carried out on skin samples. Other skin specimens were used for autoradiography with 10- and 15-μ sections exposed to Kodak *A.R.* 10 and *A.R.* 50 autoradiographic film after eosin staining. Parallel control experiments were employed with one rat receiving no therapy and three rats to which had been given DL-cystine–sulphur-35 (specific activity

31 μc./m.mole) in respective doses of 100, 10 and 1 μc. per 100 gm. weight on three successive days before killing.

In the second experiment a 10 per cent preparation of the radioactive griseofulvin was applied to an area of the skin surface of 2 normal patients and to the tinea lesions of 8 infected human patients. Geiger counting was done after application and 8 hr. later immediately prior to skin biopsy following cleansing of the area with spirit. Biopsies were examined by autoradiography.

In the first experiment with normal rats autoradiographs showed that in the griseofulvin-treated animals sites of radioactivity were limited almost entirely to the hair follicles; some accumulation was seen in the stratum corneum adjacent to the follicular openings. Of the hair follicles, only those in the anagen phase of the growth-cycle contained radioactive grains, and of these, only 1 in 10. Grains accumulated in the suprabulbar region initially, and with the growth of hair keratin, were gradually carried toward the surface, which they reached in three days. Three separate bands could be seen, interrupted by zones without activity, apparently representing the three separate doses of radioactive material given. No grains were observed in the bulb of the hair. The greater suprabulbar accumulation of radioactivity was seen in the rat killed 7 hr. after the last dose of griseofulvin.

In the rats to which DL-cystine–sulphur-35 had been given almost all the hair follicles except those in the telogen phase were found to contain radioactivity. Grain concentration was dense, in contrast to that seen in the griseofulvin-treated rats. Radioactive material had accumulated in the suprabulbar portion of the follicle, the keratogenous zone, and throughout the length of the hair shaft, suggesting that the radioactive cystine had been fully incorporated into the keratin. The stratum corneum also contained a diffuse and moderate concentration of radioactivity.

Assays based on content of radioactive chloride and radioactive griseofulvin (Table 1) agree closely, indicating that the griseofulvin–chlorine-36 molecule remained relatively intact during its 3-day sojourn in the skin examined here. One of the sets of figures obtained from spectrophotometric analysis corroborates this. Both results also suggest an increase in concentration of griseofulvin in the skin 4–8 hr. after dosage. Comparison of these figures with those obtained by calculation of the theoretical skin content

Table 1

	Treatment administered						
	Griseofulvin–^{36}Cl		DL-Cystine–^{35}S			Nil	
	1 (killed 68 hr.)	2 (killed 71 hr.)	3	4	5	6	7
Total dose of radioactivity (μc./100 gm. rat)	0·2	0·2	300	30	3	0	0
Radioactivity of skin (% of dose)	0·004	0·02	0·4	0·2	0·23	0	0
Griseofulvin assay Spectrophotometry (% of dose)	0·023	0·027					
Radioassay* (% of dose)							
Expected content based on even tissue distribution (% of dose)	0·004	0·021 0·0027					

* Assuming all chlorine-36 remains in griseofulvin.

Fig. 1. Autoradiograph showing the accumulation of griseofulvin-chlorine-36 around an infected hair. The bulk of radioactivity is in the keratogenous zone with grains located in the hair shaft of this and the adjacent hair. (× 250)

if distribution were equal throughout all tissues suggests that the concentration of griseofulvin actually in skin is approximately 10 times that expected.

Autoradiographs obtained in the second experiment demonstrated a denser collection of radioactivity in infected stratum corneum as well as in involved hair follicles. Normal skin showed a pattern of accumulation similar to that seen in uninfected rat skin, and similarly scant. In skin from lesions, however, grains were detected in almost all infected hair follicles, in the suprabulbar and keratogenous zones. Clumps of grains were also seen in infected stratum corneum, extending as far as the stratum granulosum. In a few cases in which parallel periodic acid–Schiff stains were used, the collections coincided with the areas of direct fungal infection. As may be seen (Table 2) direct counts over the lesions showed a higher concentration of radioactivity in these areas than in normal skin (10–20 per cent as contrasted with 2 per cent).

Table 2

Test area of skin	No. of cases	Radioactivity (expressed as per cent of initial value)
Normal	2	1
Infected	8	9–18 (average 12)

The similarity in the distribution of griseofulvin-chlorine-36 and DL-cystine–sulphur-35 in the hair follicles of the rat suggests that the former is taken up during the production of keratin. That it is not intimately involved in the manufacture of keratin is indicated by the limitation of its distribution to the actively growing phase, its interrupted, band-like localization in the hair keratin, and finally, by its failure to appear in quantity in the soft keratin of the stratum corneum. In all these points it differs from radioactive cystine. Studies with radioactive glucose and anions[1] have shown that these are localized in the hair bulb in contrast to those materials directly concerned with keratin. Based on this experience our findings would indicate that the griseofulvin-chlorine-36 molecule remained relatively intact, since our distribution of radioactivity resembled that observed for cations and amino-acids involved in keratin production. This conclusion is supported

by the results in the spectrophotometric and radio-assays, which also indicate a preferential localization of griseofulvin in keratin sites.

Since the radioactive material did not persist in one site but was carried distally with the keratin, there does not appear to be any intracellular fixation, but rather an incorporation in the interstices of the keratin 'lattice'[2] described by Rudall. This evidence supports the findings of Gentles[3] in which he demonstrated a large water-extractable moiety in skin containing griseofulvin. My failure to demonstrate radioactivity in any quiescent areas of skin suggests the possible inability of griseofulvin to affect fungus situated in necrotic tissue. In work on human skin, griseofulvin was found to concentrate preferentially in infected areas and particularly around sites of fungal accumulation. This might indicate that the associated partial degeneration of infected keratin favours the entrance of griseofulvin into the keratin interstices where its fixation permits a fungistatic action.

I wish to thank the Glaxo Laboratories, Ltd., Greenford, Middx., which supplied the radioactive griseofulvin and carried out the assay of that material.

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BIOLOGY

Development of Mouse Eggs beneath the Kidney Capsule

During experiments to produce extra-uterine pregnancies in mice, eggs were transplanted beneath the kidney capsule. Only one egg was transplanted under any one kidney capsule in each experiment. The eggs used were 1-, 2- and 4-cell stages, morulæ and blastocysts.

The transplants were made within a pure line, and within a heterogeneous population. Males and pregnant and non-pregnant females were used as hosts. Results are shown in Table 1.

Table 1

Stage of egg	No. of experiments	No. of positive results	No. of embryos *x*	No. of *T.G.C.* and *E.M. y*
1 cell	10	8	0	8
2 cell	8	6	0	6
4 cell	8	6	0	6
Morulæ	10	7	0	7
Blastocysts	50	41	16	25

Embryos *x* = No. of experiments showing embryonic shield derivatives.
T.G.C. and E.M. y = No. of experiments showing embryonic membranes and trophoblastic giant cells.

All 36 pre-uterine eggs extracted from the oviduct failed to to produce embryonic-shield derivatives. Fawcett[1], in a similar experiment, transplanted 4–8-cell stage eggs only, and also stated that "differentiation of recognizable tissues or organs of the embryo proper did not occur". In contrast to this result, 16 of the 50 eggs (blastocysts) obtained from the uterus and transferred beneath the kidney capsule gave rise to embryonic shield derivatives. In a number of cases perfectly normal embryos developed (Figs. 1 and 2).

Fig. 1. Sagittal section of embryo embedded in kidney cortex of adult male mouse. Autopsy four days after transplantation of one blastocyst

Fig. 2. Section of embryo at head-fold stage with all the embryonic membranes. Autopsy nine days after transplantation of one blastocyst

Nicholas[2], using the rat as experimental animal, transplanted eggs of various stages to the same site and found that only disorganized embryonic tissues developed, and that there was no invasion of the host kidney. In my experiments the embryonic tissues were always surrounded by a matrix of highly invasive trophoblastic giant cells, which caused considerable destruction of the kidney. Lacunæ of extravasated maternal blood lie in the interstices of this matrix of giant cells.

With the progress of invasion of the kidney, the embryonic tissue sinks deeper below the original level of the cortical surface. Compared with intra-uterine

gestation, embryos beneath the kidney-capsule are usually retarded in development. Genotype or sex of the host had no effect on the growth of embryonic tissues, except that in transplants to pregnant females the mass of embryonic tissue tended to be larger, but not more differentiated.

I wish to acknowledge with thanks the help given to me by Dr. M. Fischberg. This work was carried out during the tenure of a Research Studentship of the Department of Scientific and Industrial Research.

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Cyclical Changes in the Distribution of the Testis Lipids in the Common Frog, *Rana temporaria*

That the cytoplasm of the interstitial cells contains fatty substances has been known since as early as 1850 when Leydig reported them in the interstitium of bat, pig, cat and mole[1]. Since then, Leydig cell lipids have been reported in every group from the Agnatha to the Mammalia[2]. Although an enormous amount of work has been carried out on the seminiferous tubules, the majority of workers have used traditional wax-embedding techniques which result in the dissolution of lipids. Thus many aspects of the testis cycle escaped notice until comparatively recent times[3]. It is now known that at the conclusion of spermatogenesis in wild birds, the seminiferous tubules undergo a metamorphosis involving the sudden genesis of large quantities of cholesterol-positive lipids. At about the same time a new generation of Leydig cells arises in the exhausted interstitium[4]. This tubule lipid appears to contain progestogens[5]. Such a complete post-nuptial tubule metamorphosis does not occur in seasonal mammals[6], but it is a regular part of the cycle of certain teleost fishes[7] and has been reported in at least one reptile[8]. It is not known whether Amphibia display comparable events in the seminiferous and endocrine elements of the testis. Few, however, have been studied to this end.

In order to investigate this phenomenon adult male grass frogs (*Rana temporaria*) were collected during every month for one year and the testicular material subjected to a series of histological and histochemical techniques[9].

R. temporaria breeds, in Great Britain, during late February and early March, and specimens killed during this period can be classified into two main groups based on the sudanophil condition of the interstitium and the spermatogenetic condition of the tubules: (1) Those specimens in which sperm shedding has not yet occurred possess tubules filled with bunched and free spermatozoa, scarcely any intra-tubular lipid material and no cholesterol. The interstitium, on the other hand, contains Leydig cells the cytoplasm of which displays numerous lipoidal droplets that give a positive cholesterol reaction when subjected to the Schultz test[10]. Numerous fuchsinophil granules are also present in these cells. (2) Specimens that have discharged their spermatozoa and the tubules of which now possess only spermatogonia and Sertoli cells contain some

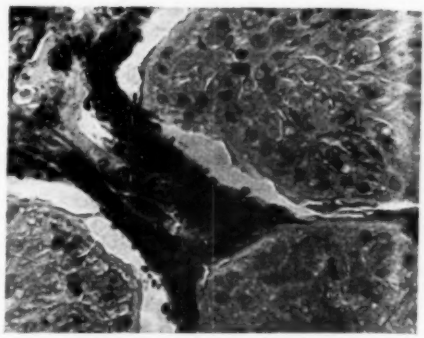

Fig. 1. Frog testis in early March. The 'spent' tubules have not yet become lipoidal but the interstitial Leydig cells are filled with dense cholesterol-positive lipids. (Formol–calcium fixation, sudan-black colouring at 8μ. × c. 260)

intra-tubular lipids in the form of a few small Schultz-negative droplets which appear to be in the Sertoli cytoplasm. The interstitial cells are now densely lipoidal and strongly cholesterol-positive (Fig. 1). Fuchsinophil granules have also increased in number. In addition to these two main categories, some autopsied specimens show an intermediate stage in which only some of the testis tubules have discharged their spermatozoa while others still retain theirs. In such gonads the interstitium adjacent to the 'spent' tubules is much more densely filled with cholesterol-rich lipid material than tissue surrounding sperm-containing elements.

As the breeding season progresses, more and more frogs discharge their ripened germinal products and undergo the above-described sequence of events, so that by late March and early April the majority of specimens have passed into their post-nuptial phase and possess testes with Leydig cells in maximum lipid condition and with nuclei measuring 10μ × 6μ. From then until June prespermatogenesis occurs, during which multiplication of spermatogonia takes place, but these do not develop further than into primary spermatocytes, which thereupon soon degenerate[11,12]. Major changes in the lipid distribution also occur during this period. Leydig cells gradually lose their cytoplasmic cholesterol-rich lipids and fuchsinophil granules, and, accompanying this, there is a shrinkage of the nucleus to an average size of 6μ × 4μ. In the adjacent seminiferous elements,

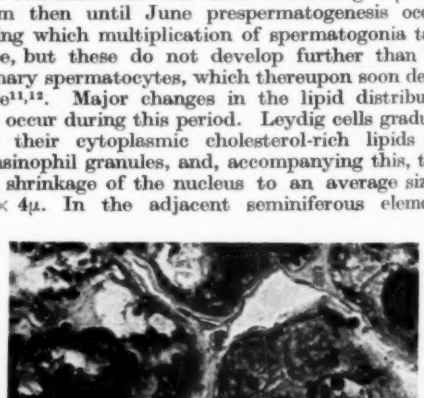

Fig. 2. Frog testis in June. Tubules are now densely lipoidal and cholesterol-positive. Interstitial lipids have disappeared. (Technique and magnification as in Fig. 1)

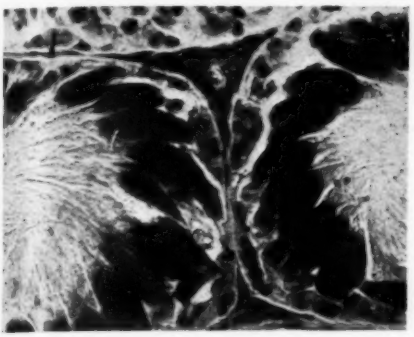

Fig. 3. Frog testis in December. Bunched spermatozoa in tubules. Interstitial Leydig cells have only a few small lipid droplets in their cytoplasm. (Technique and magnification as in Fig. 1)

on the other hand, there is a rapid build-up of intra-tubular lipid. By late June they have increased in size and contain densely lipoidal cytoplasm which now gives a strong cholesterol reaction to histochemical tests. When this Sertoli steatogenesis has reached its maximum level, Leydig cells have lost nearly all lipoidal inclusions (Fig. 2) and become indistinguishable from ordinary connective tissue cells, a condition previously noted by Aron[12]. A few interstitial cell mitoses are seen during this period.

Throughout July and August the tubule lipids disappear and spermatogenesis restarts. This proceeds rapidly and frogs killed in September have gonads in which the seminiferous tubules are once again filled with bunched spermatozoa and contain only small traces of the once profuse intra-tubular lipid material. In the interstitium, Leydig cells are once again starting to develop. This is the so-called static phase[13] during which hibernation occurs. Tubules are at their maximum development and will remain unchanged throughout the winter until sperm discharge in the following breeding season. Although sometimes described as a period of complete testicular inactivity[11], interstitial development does continue. This is revealed by the gradual accumulation of fuchsinophil granules in the Leydig cells, which become more and more numerous during the months of hibernation. The nucleus also increases in size until it once again measures 9μ × 6μ. A few small lipid droplets appear in December (Fig. 3) and by January the majority of Leydig cells contain numerous small lipid droplets in their cytoplasm. Cholesterol is also present from December onwards. In February there is a rapid build-up of interstitial lipid and the animal is once again ready to breed.

These observations reveal that the seminiferous tubules of *R. temporaria* undergo a sequence of cyclical events comparable with those occurring in other sub-mammalian vertebrates. This involves a post-nuptial accumulation of cholesterol-positive lipid within the tubules, and its disappearance when spermatogenesis restarts. This may be of endocrine significance since analysis of similar material in birds has shown it to contain progestogens[5]. The presence of tubule lipid coincides with the period of prespermatogenesis. The interstitial cycle, too, is basically similar to that of birds in that it involves a seasonal accumulation and discharge of cholesterol-positive lipids. But whereas in birds and fishes the Leydig cells gradually discharge the sudanophil contents before mating, the interstitium of the frog contains its maximum lipid content immediately after mating and then becomes slowly exhausted. A similar cycle occurs in seasonal mammals[6]. These events led Champy[11] to suggest that the interstitium of *Rana* was not involved in the development of the secondary sexual characters. However, the sudden increase in lipid and cholesterol in the Leydig cells after sperm shedding does not necessarily imply that the cholesterol is being utilized. On the contrary, it seems not impossible that this phenomenon may indicate that sex hormone secretion, having ceased after copulation, cholesterol is no longer being used in its manufacture, and accumulates. This explanation is supported by the work of Mershall and Wilkinson[14]. These authors found that Orkney voles (*Microtus orcadensis*) seasonally expend their Leydig lipids in apparently the same manner as birds, but that when solitary male voles were placed in complete darkness the cholesterol-positive lipids were no longer used but were stored in the interstitium.

The possible endocrine significance of these events is being investigated by means of chromatography and bio-assay techniques and will be reported elsewhere.

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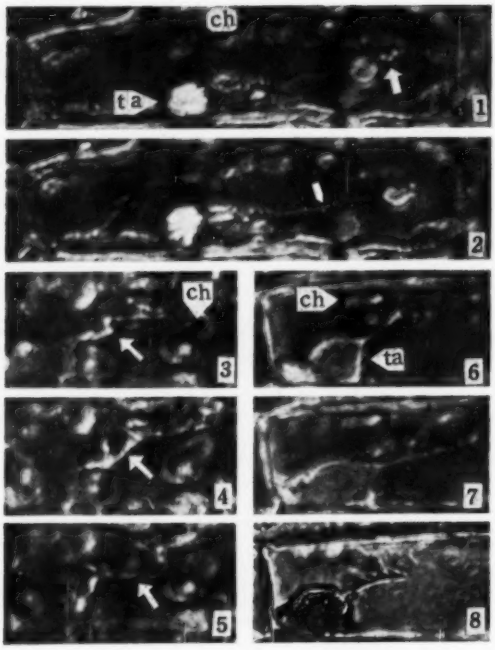
Behaviour of a Thread-like Apparatus in the Petiole of *Mimosa pudica*

Although I have published several communications[1–5] concerning the cytology of the thread-like apparatus in *Mimosa pudica*, these observations were carried out with vital staining and/or with fixation. For a full understanding of the subject, it is necessary to observe the non-treated material in the living state. In this communication, the behaviour of the thread-like apparatus with a phase-contrast microscope is reported.

The sections of the fresh material were prepared in the following manner. Petiole sections were cut 30–50μ in thickness by a hand microtome and by razor, and then sections were immersed in medium solution. All sections were freshly prepared at the beginning of each observation. Observations were made with phase-contrast microscopy in conjunction with dark-medium contrast 100 × objective with a 'Periplan' 10 × ocular. Photomicrographs were



Figs. 1–7. Photomicrographs of the thread-like apparatus of lacunal parenchymatous cells of *Mimosa* petiole, taken with the phase microscope. 1, arrow indicates rosary-like portion; 2, small arrow indicates tiny granule in vicinity of thread; 3-5, vermiculation of a part of thread indicated by arrows; 6, 7, connexion of tannin vacuole and thread-like apparatus. 1–7 are mounted in tap water. 8, caffeine reaction in the same cell of Fig. 7 with bright field microscope (× about 800). *ch*, chloroplast; *ta*, tannin vacuole

taken with a 35-mm. camera attachment (Figs. 1–7). The material in the present work consisted chiefly of those plants which had already received a stimulus. All experiments were carried out at room temperature (25–30° C.) in the summer of 1959.

In the case of the present observations, we must discriminate carefully between the thread-like apparatus and plasma streaming. Furthermore, it is of fundamental importance to distinguish the thread-like apparatus from the other cellular elements. I have attempted the caffeine treatment on the intact cell on the basis of past experience. The reaction of precipitation in the presence of caffeine occurred as one of the specifications of the thread-like apparatus, as was shown previously[1]. The reason for this is simply that caffeine solution was used as a medium after the successful observation of the behaviour of the thread-like apparatus.

The sections were mounted in tap water, without any subsequent treatment, a method deemed most harmless to the protoplasm. In one of the lacunal parenchymatous cells, several minutes after preparation, a movement of the thread-like apparatus began to appear (Figs. 1 and 2). The thread-like apparatus assumed many shapes. A part of the thread is shown as a rosary-like figure or small mass indicated by arrow in Fig. 1, and other parts of the thread are developed to such an extent as to form a long slender structure. Each portion of the thread moves continuously. On careful examination the threads, though not clear in these photographs, are seen to be accompanied by tiny granules. These tiny granules are difficult to photograph in living cells, due to their small size and rapid motion along the thread-like apparatus. But as shown in Fig. 2, successful photographs exhibit a small number of these granules near the thread. The small arrow indicates a tiny granule.

For the purpose of gaining a more complete knowledge of the behaviour of the thread-like apparatus, I made detailed observations on the threads and other cellular elements. The material was soaked in tap-water as mentioned in the foregoing experiment. As shown in the series of photomicrographs (Figs. 3, 4 and 5), the vermiculation of a part of the thread-like apparatus is observable. This vermiculation occurred continuously.

Next, I sought a connexion between the thread-like apparatus and tannin vacuole. The series of photomicrographs show that the thread extends from the tannin vacuole as a horn or a tail (Figs. 6, 7 and 8). In this case, the terminal of the thread was associated with the chloroplasts which were accompanied by tiny granules. Furthermore, caffeine treatment with bright field microscopy was also undertaken (Fig. 8). Accordingly, from the above observation, it seems that the thread-like apparatus is a modified vacuome system of the tannin vacuole.

Recently, Sorokin[6] observed the long filamentous structure in living epidermal cells of the Cichoriese. These filaments branch out, and even anastomose, forming loops and networks under anaerobic conditions. They are regarded as modified plastids. The investigation and the critical comparison of the cellular elements and thread-like apparatus in *Mimosa* must be the subject of future work. The details will be reported in the near future.

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Differentiation in *Peronospora tabacina* Adam.

Some results that we have obtained indicate that at least three ecotypes of *Peronospora tabacina* occur in Australia and that the differences between them may prove of importance in breeding for resistance to the pathogen.

Zink[1] reported the occurrence of two races of *P. effusa* (Grev. ex Desm.) Ces. on spinach, and Grabe and Dunleavy[2] described eight races of *P. manshurica* (Naum.) Syd. ex Gaum. on soybeans. Differences between the host-ranges of *P. tabacina* of tobacco in Australia and the United States were reported by Hill[3], who later[4] suggested the possibility of different strains of the pathogen. Within Australia the relative isolation geographically of the major tobacco-growing areas could provide conditions for the development of distinct strains of *P. tabacina*.

On two occasions comparable seedling populations of tobacco breeding lines with some resistance to attack by *P. tabacina* were spray-inoculated with standardized spore suspensions of multispore isolates of *P. tabacina* from North Queensland and Canberra under similar glasshouse conditions. The Canberra isolate was originally introduced from Victoria in

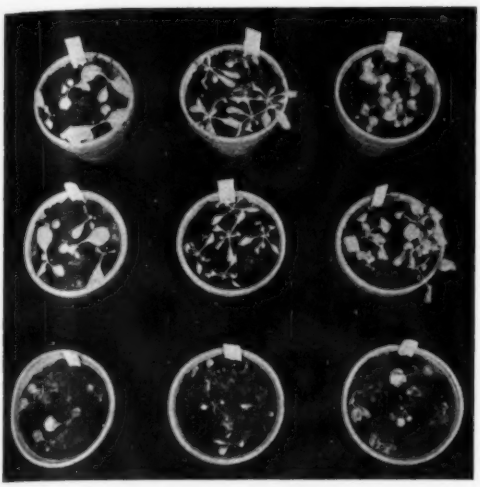

Fig. 1. Disease reaction of three *Nicotiana* spp. to isolates of *P. tabacina* from: front row, Canberra; middle row, North Queensland; back row, Western Australia. The species are: left, *tabacum* L. (var. Virginia Gold); middle, *trigonophylla* Dunal; right, *umbratica*, N. T. Burbidge. Photo 12 days after inoculation

1954. Seedlings showing sporulating lesions within the first 14 days after inoculation were classed as susceptible. The numbers of resistant and susceptible plants in these tests are shown in Table 1. A higher proportion of the plants was susceptible to the Canberra isolates than to the North Queensland isolates, and this was true for all 7 lines which included resistance derived from 3 species.

Groups of four-week-old glasshouse seedlings of 13 spp. of *Nicotiana* which had been obtained as seeds from 33 different localities were inoculated with the two isolates of *P. tabacina* described above and a third isolate from Western Australia. At 7 days after inoculation the amount of sporulation on seedlings grown from the same seed lot was similar for all three collections of the pathogen. In seedlings from 19 seed localities a similar response to infection was maintained as the disease developed with time. In the remaining 14 lots (including *N. tabacum* var. Virginia Gold) the subsequent incidence of disease in the four tests, as recorded after 12 and 20 days and summarized in Table 2, reflected differences due to source of inoculum (Fig. 1).

Table 1. Numbers of Tobacco Seedlings Resistant and Susceptible to Isolates of *P. tabacina* from North Queensland (N.Q.) and Canberra (C.)

Breeding line	Source of resistance	Source of inoculum			
		N.Q.		C.	
		R	*S*	*R*	*S*
Test 1					
1	*N. debneyi*	72	0	58	14
2	*N. knightiana*	72	0	55	15
3	unknown	648	0	547	107
4	*N. goodspeedii*	15	1	0	16
5	*N. goodspeedii*	9	3	0	12
Test 2					
6	*N. goodspeedii*	100	116	0	216
7	*N. debneyi*	88	128	0	216

Table 2. Disease Severity in 14 Lines of *Nicotiana* spp.

Number of seedling lines	Incidence of disease in decreasing order of severity			
2	W.A.	C.	or	N.Q.
5	C.	N.Q.	or	W.A.
6	C.	N.Q.		W.A.
1	C.	W.A.		N.Q.

W.A., Western Australia; C., Canberra; N.Q., North Queensland.

Sporulation intensity of the three isolates on *N. tabacum* var. Virginia Gold was determined using the leaf disk technique of Cruickshank and Müller[5]. In two separate tests six disk replications, taken from leaves of plants inoculated with each isolate, were incubated at 20° C. For comparative purposes the Canberra isolate was given a sporulation intensity index value of 100, the corresponding values for the North Queensland and Western Australian isolates being 44·1 and 54·9 respectively. Thus the number of conidia produced by the Canberra isolate was approximately twice that produced under similar conditions by each of the other two isolates.

These results indicate that isolates of *P. tabacina* differ in their pathogenicity range, host reaction and reproductive capacity. The first of these differences, although not so clearly defined at present as those reported for other *Peronospora* spp.[1,2] represents qualitative differences which may be of considerable importance in the development of blue-mould (*P. tabacina*) resistant tobacco varieties. The second and third differences are quantitative only but may be of significance in terms of epidemiology. Environmental conditions favouring spread of the disease occur only periodically, therefore a reduced capacity for reproduction could result in a much slower build-up of the pathogen and a significant reduction in loss due to the disease.

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CYTOLOGY

Uptake of Heparin by Cells

In a previous communication[1] we showed that the epithelial cells and lymphocytes of the thymus gland are capable of being converted into mast cells under conditions of tissue proliferation. With the view of confirming the correctness of this finding, we added partially decomposed heparin in cultures of thymus, spleen and lymph-node tissues and found that the heparinoid substance was taken up considerably by the thymus and by the other two tissues to a lesser extent. The heparinoid matter is then synthesized into complete metachromatic substance in the cells[2]. Tissues obtained from new-born to 1-month-old rats were used in those experiments. We have now attempted to ascertain whether there exists such a difference between the various tissues also in respect of heparin which has not been decomposed and to observe the behaviour of the different tissues in respect to their readiness to take up heparin in the successive phases of ontogenesis.

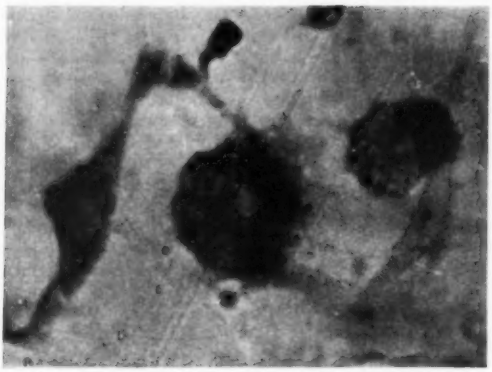

Fig. 1. Cells containing heparin in the culture of thymic tissue taken from 16–17-day-old rat embryo. Toluidine blue. (*c.* × 400)

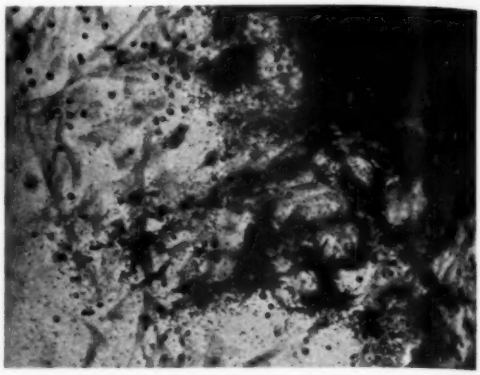

Fig. 3. Formation and disintegration of regular mast cells in the migration zone of the thymus obtained from pregnant rat. Toluidine blue. (× 700)

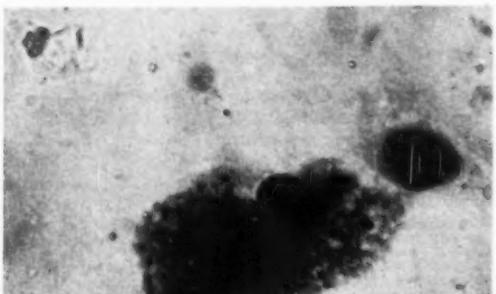

Fig. 2. Cell containing heparin in the thymus of rat weighing 200 gm. Intracellular heparin appears in the shape of coarse lumps. Toluidine blue. (× *c.* 400)

Fig. 4. Enlarged detail of Fig. 3. Toluidine blue. (× 700)

Tissues taken from the spleen, liver, lymph nodes, heart, kidney and thymus of 16-day-old intra-uterine and new-born rats weighing 50 gm., 100 gm., 150 gm. and 200 gm. respectively, and also pregnant and tumorous rats were explanted to Maximow's double cover-slip cultures and grown on a medium consisting of hen plasma coagulated with chick embryo extract. A mixture of horse serum, Tyrode solution and chick embryo extract (3 : 6 : 1) served as bathing fluid. The cultures were first washed 48 hr. after the explantation and again after another 24 hr. The fluid used for this second washing also contained 1 mgm. heparin per 1 ml. of the fluid (heparin pulvis Richter 80 I.U./mgm.). The cultures were fixed in Carnoy's fixing fluid and stained with toluidine blue 48 hr. after the heparin treatment. A total of 800 cultures was divided into two groups.

Experimental results are given in Table 1. The number of cells containing heparin (determined by subjective estimates) is indicated by the number of crosses.

It is evident that all embryonic tissues examined are capable of taking up heparin; kidney and heart of new-born rats are no longer able to do so, and the liver loses this capacity in animals weighing 100 gm. Thymus, spleen and lymph nodes seem to be able to take up heparin throughout the series, and this ability is especially pronounced in the case of the thymus. It can be concluded that all tissues of an embryo, the organs of which contain a great amount of mucopolysaccharides, are capable of taking up heparin, and that—with advancing age—this function is limited to the lymphatic organs, and to the thymus in the first place.

The form in which heparin appears varies according to the age of the animal from which the tissues are taken. Dispersion of heparin granules is very high in the cells of young tissues, and granulation becomes coarser and coarser with advancing age. We regard

Table 1. Uptake of Heparin at Different Stages of the Rat's Ontogenesis

Tissue	Embryo	New-born	50 gm.	100 gm.	150 gm.	200 gm.	Pregnant	Tumorous
Spleen	+	+	++	+	+ ?	+ ?	+ ?	–
Liver	+	+	+	–	–	–	–	–
Lymph node	–	+	++	++	+	+	++	+ ?
Myocardium	+	–	–	–	–	–	–	–
Kidney	+	–	–	–	–	–	–	–
Thymus	++	++	+++	+++	+++	++	+++	+ ?

this phenomenon as being more or less in harmony with intracellular colloid-chemical alterations associated with senescence[2–5].

Especial interest is attached to tissues collected from tumorous and pregnant animals. One encounters in them not merely mast-cell-like cells containing heparin but also regular mast cells which disintegrate into granules—a process similar to the disintegration of mast cells contained in the connective tissue. It may be assumed that processes of tissue proliferation involve a factor in the organism which releases cellular disintegration so that, in contradiction to current belief, the break-down of mast cells is not caused by mechanical factors alone.

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Cytochemical Evidence of the Origin of Lipid Bodies (so-called Golgi Bodies) from Mitochondria

Since Hirsch's[1] theory of the Golgi presubstance, according to which there exist homogeneous granular mass as a precursor of the 'Golgi' system, there has been very little attempt to investigate the origin of these inclusions in the cell. Nath and Chopra[2] reported the origin of 'Golgi dictyosomes' from mitochondria in the spermatocytes of the slug, *Anadenus altivagus*. However, such a conclusion was arrived at on the basis of morphological study inasmuch as the 'Golgi dictyosomes' disappear at the metaphase and anaphase stages of meiosis I and II, probably merging into the mitochondria; they again appear in the respective telophase stages by the alignment of lipid granules, which seemed to take their origin from the mitochondria. These observations were later confirmed by Nath and Gupta[3] in the living spermatocytes of *Anadenus* and *Euaustenia* under phase-contrast microscopy.

My present cytochemical investigations on the developing oocytes of a large number of species of fish, namely, *Ophiocephalus punctatus*[4], *Barbus ticto*[5], *Mystus tengara*, *Cirrhina reba*, *Labeo dyocheilus*, *Chrysophrys berda*, *Muraenesox talbonides*, *Pseudo-rhombus elevatus*, *Boleophthalmus dusumerii*[6], clearly demonstrate the origin of lipid granules, corresponding to 'Golgi' granules of earlier workers, from the mitochondria. The earliest oogonia completely lack the 'Golgi' material, and consist of only a juxta-nuclear mass of fine mitochondrial granules. However, in the developing oogonia of freshwater fishes, namely, *Ophiocephalus*, *Barbus*, *Mystus*, *Cirrhina* and *Labeo*, the mitochondrial granules align themselves into filaments, while in those of the marine fishes, namely, *Chrysophrys*, *Muraenesox*, *Pseudo-rhombus*, and *Boleophthalmus*, the granular juxta-nuclear mass becomes very dense. In the former case a large number of bigger and denser lipid granules appear as tiny blebs on the surface, and in intimate association with the mitochondrial filaments; but in the latter they develop among the juxta-nuclear mass. However, these inclusions acquire a separate entity in the growing oocytes; they either grow directly into large spheres or attach themselves to tiny vacuoles in the cytoplasm so as to produce rings and crescents.

These 'Golgi' inclusions are very rich in lipid material as revealed by their strong coloration in sudan black *B*. Baker's[7] acid-hæmatin technique with pyridine extraction control reveals the phospholipid nature of the lipid material. However, it changes later into triglycerides as shown by Cain's nile blue technique. The pure lipid nature of these inclusions is demonstrated by the lipid extraction techniques, and is further confirmed by the absence of carbohydrates and proteins, as revealed by periodic acid–Schiff reagent, and Mazia's mercuric bromphenol blue tests, respectively.

Thus it may be concluded from the above results that the mitochondria which always have some phospholipid material in definite configuration with proteins are constantly pinching off phospholipid material in the form of 'Golgi' granules on their surface. The extrusion of lipid material by the mitochondria has also been found by Lever[9] by electron microscopy. Therefore, it seems very probable that the mitochondria also produce 'Golgi' material in the cell, besides their vital oxidative activities.

It may also be stated that the 'Golgi' material always appears in the form of granules in the early stages which may later grow directly into big spheres, or they may come in contact with vacuoles and spread around them to form rings and crescents showing the characteristic duplex structure corresponding to the externum and internum of Hirsch. However, in some cells where 'Golgi' granules lie close together, osmium and silver impregnation tends to make a 'net-work'-like pattern, which is soon resolved in the growing oocytes by their dispersal. These conclusions support Baker's[10] new terminology 'lipochondria' for 'Golgi' inclusions, which would not only signify their granular form and lipid nature but would also indicate their mitochondrial origin.

I wish to thank Prof. Vishwa Nath and Dr. H. S. Roberts for helpful suggestions.

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VIROLOGY

Activation of One Plant Virus by Another

Tobacco necrosis is a disease that can be caused by several serologically unrelated viruses[1], all of which are soil-borne and cause necrotic local lesions in leaves of tobacco (*Nicotiana tabacum* L.) and French bean (*Phaseolus vulgaris* L.) plants. The virus Bawden and Pirie[2,3] called the 'Rothamsted culture'

differed in several respects from other members of the group; the ratio of infectivity to specific antigen content varied greatly in different preparations, it lost infectivity *in vitro* more rapidly than the others, and infective preparations always contained particles of two different sizes, about 17 mμ and about 30 mμ in diameter. Crystalline preparations (identified as nucleoproteins), consisting almost entirely of the smaller particles, had little or no infectivity, whereas other partly purified preparations that contained many more of the larger particles were infective. From this, Bawden and Pirie concluded that probably only the larger particles were infective and that the smaller ones, which were usually the major constituent of their preparations, were an additional product of the multiplication of the larger ones[4].

We have now found that the large and small particles in the Rothamsted tobacco necrosis are serologically unrelated, and that whereas the large particles can apparently multiply by themselves, the small ones multiply only in the presence of the large. Particles having different sedimentation-rates were separated from partly purified virus preparations, made as described by Bawden and Pirie[5], by centrifuging in sucrose density gradients[6]. After sedimenting for 2 hr. at 17,000 r.p.m. (about 25,000*g* at the top of the gradient column), two and sometimes three light-scattering zones were formed. The top zone contained particles 17 mμ in diameter, the second one particles 30 mμ in diameter, and the third zone, when present, contained aggregates each apparently consisting of a group of 12 of the 17 mμ particles. The approximate sedimentation constants, estimated from the depths of the zones as described elsewhere[7], were 45*S*, 116*S*, and 216*S*, respectively, assuming the value for tobacco mosaic virus to be 187*S*. These three components presumably correspond to those reported as having sedimentation constants of 51, 116 and 240*S* (ref. 2).

When material from each of these zones was inoculated separately to French bean or tobacco plants, only that from the middle zone produced lesions, and these were more uniform in size than those produced from inocula made by extracting sap from diseased plants. In one experiment, when these leaves inoculated from the middle zone were later macerated and the virus from them extracted and purified, the ratio of 30 mμ to 17 mμ particles was 21 : 1. Similar leaves, inoculated with a mixture of equal volumes of top zone and middle zone material, gave a ratio of 1 : 1. Leaves inoculated with either top or bottom zone material alone failed to develop lesions, and extracts from such leaves showed no virus-like particles when examined with the electron microscope. When tested in double-diffusion precipitin tests in agar gel against an antiserum containing antibodies to both large and small particles, the extracts from plants inoculated with middle zone material gave one line, those from plants inoculated with mixtures of top and middle zone gave two lines, and those from plants inoculated separately with top or bottom zone only gave no lines.

Table 1 shows the effects of using as inocula particles from the middle and top zones. Mixing the two decreases the size of the lesions below that produced by the middle zone alone.

A small proportion of small particles did not alter the total number of lesions, but only a few were now large. Further successive increases in the proportion of small particles in the inoculum did decrease the total number of lesions, and almost all were small.

Table 1. Infectivity of Small and Large Particles separately and when mixed in Different Proportions

Inocula	Numbers of lesions on seven half-leaves of French beans	
	Large lesions	Small lesions
Large particles, 4 mgm./l.	322	0
Large particles, 4 mgm./ml. + small, 5 mgm./l.	12	314
Large particles, 4 mgm./ml. + small, 20 mgm./l.	4	295
Large particles, 4 mgm./ml. + small, 80 mgm./l.	1	184
Small particles, 80 mgm./l.	0	0

These results indicate that the small particles interfere with, and when abundant may even suppress, the multiplication of the large particles, and that they multiply detectably only when inoculated together with the large ones. It seems likely that a pure preparation of large particles would infect and multiply without producing any of the small particles, but it is impossible to establish this because the middle zone cannot be removed from the centrifuge tube without some contamination from top or bottom zones. However, our results clearly suggest that the number of small particles produced in the inoculated leaves depends upon the proportion of small particles in the inoculum.

The production of non-infective particles along with infective ones has been described before with other viruses, but in all such examples known to us the non-infective particles are serologically related to the infective ones so that they can be regarded as products of the synthesis of one virus. The association of two sizes of particles in the Rothamsted tobacco necrosis culture appears to be unique, for the two kinds of particles are serologically unrelated and thus seem to be separate viruses, one of which is apparently completely dependent upon the other for its multiplication.

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Infective Ribonucleic Acid from the Viruses of Talfan and Teschen Diseases

Infective ribonucleic acids have been obtained from several animal viruses by the cold phenol method described by Gierer and Schramm[1]. In general, however, most of the experiments have been done with crude suspensions, and it has not always been clear whether the infective ribonucleic acid was derived from the virus itself or from a viral precursor or other related material. The work of Huppert and Sanders[2] with mouse encephalomyocarditis virus has shown clearly that the infective ribonucleic acid which can be obtained from mouse carcinoma cells infected with this virus has its origin in a viral

precursor and not in the virus itself. In fact, infective ribonucleic acid cannot be extracted from this virus by the cold phenol method. On the other hand, it is likely that the infective material which can be obtained from suspensions of tissues infected with the viruses of poliomyelitis[3], mouse encephalomyelitis[4], Mengo encephalitis[5], Murray Valley encephalitis[6] and foot-and-mouth disease[7,8] by this method is being derived from the viruses themselves. This difference in behaviour emphasizes the differences in the chemical structures of the viruses. Furthermore, the work of Wecker[9] has shown that purified Western equine encephalomyelitis virus, which does not yield any infective ribonucleic acid on treatment with cold phenol, can be made to do so if the extraction is carried out at 40–50° C.

Similar experiments have now been done with the viruses of Talfan and Teschen diseases. Talfan disease is a recently described[10] polioencephalomyelitis affecting young pigs in Britain and shows close similarities to Teschen disease, an infection of pigs well known in Europe. The cross-neutralization tests described by Chaproniere, Done and Andrewes[11] suggested that the two diseases are caused by identical or similar viruses. The preliminary results described here show that the infective material which can be obtained from suspensions of each virus by treatment with cold phenol is a ribonucleic acid which is being extracted either from the virus itself or from a material which sediments along with the virus in the ultracentrifuge.

Tissue culture fluids were harvested from pig kidney cell monolayers 24–30 hr. after infection. After this period of incubation, all the cells were off the glass and the virus titre was at a maximum. The virus suspensions were concentrated by dialysis against polyethylene glycol[12] at 2° C. These concentrates were then centrifuged at 30,000 r.p.m. for 1 hr. in the *L* 30·2 head of the 'Spinco' ultracentrifuge. The top 7 ml. (supernatant fraction) of each tube was then removed and the pellet suspended in the lower 2 ml. diluted with a further 7 ml. of tissue culture medium (deposit fraction). The virus content of the initial concentrate and of each fraction was determined by observing the number of plaques formed on pig kidney cell monolayers. Tenfold dilutions were used, and the monolayers stained with neutral red after 96 hr. incubation. Phenol extracts were prepared from each fraction and 0·4 ml. aliquots inoculated on to pig kidney cell monolayers in 4-oz. bottles. Four bottles per tenfold dilution were used and the monolayers were examined daily for cytopathic effects. The results (Table 1) show that infective ribonucleic acid could not be detected in the phenol extracts of the supernatant fractions but only in the deposit fractions. This is similar to the results we have obtained previously with foot-and-mouth disease virus[7], but is in direct contrast to those obtained with mouse encephalomyocarditis virus[2]. In the latter case, infective ribonucleic acid could be obtained from the supernatant fraction only, although this contained less than 0·1 per cent of the initial virus.

The cytopathic effects produced by the phenol extracts could be prevented by incubating them with 10 μgm. ribonuclease/ml. for 15 min. at 20° C. prior to inoculation on to the monolayers. As the titre of the parent viruses was not affected by adding 10 times this amount of the enzyme to each virus dilution, it seems probable that the infective agent in the phenol extracts is a ribonucleic acid.

Table 1. YIELD OF INFECTIVE RIBONUCLEIC ACID FROM ULTRACENTRIFUGE FRACTIONS OF TESCHEN AND TALFAN VIRUSES

Virus	Fraction	Virus titre (p.f.u./ml.)	Ribonucleic acid titre (log *ID*50/ml.*)
Talfan	Initial	$3·6 \times 10^8$	2·2
	Supernatant	$1·0 \times 10^6$	None detected
	Deposit	$2·5 \times 10^8$	2·2
Teschen	Initial	$2·4 \times 10^8$	2·0
	Supernatant	$1·3 \times 10^5$	None detected
	Deposit	$1·5 \times 10^8$	2·2

* 0·4 ml. inocula used.

The fluids harvested from those monolayers which showed cytopathic effects following infection with the ribonucleic acid had properties similar to those of the intact viruses. They were neutralized by antisera prepared by inoculation of the viruses into rabbits. Thus 100 *ID*50 of the parent virus or the ribonucleic acid harvest was neutralized by an equal volume of 1/1,000 homologous rabbit antiserum, as measured in pig kidney cell suspensions by the metabolic inhibition test (Martin, W. B., and Chapman, W. G., unpublished work). The possibility that the neutralization was an anti-tissue effect of the sera was rejected, as the same sera had no effect on the titration end-point of foot-and-mouth disease virus (strain 997, Waldmann type *C*) in the same cell system.

We wish to thank Mrs. J. O. Farley for the rabbit antisera and Dr. W. B. Martin and Mr. W. G. Chapman for furnishing us with details of the metabolic inhibition test prior to publication.

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Polyoma Virus Mutant with a Reduction in Tumour Formation

INFECTION with polyoma virus *in vivo* can result in a cytopathic effect, hyperplasia, and the formation of benign or of malignant tumours[1]. The use of single plaque isolations has indicated that these different types of response can be produced by the same virus[2]. During the course of investigations on the induction of different responses to virus infection *in vitro*, a plaque-type mutant of polyoma, with a smaller-sized plaque than the wild type, was found in the virus produced by a line of mouse cells with a stable cell-virus association[3]. This line of cells was established by the culture of survivors after cytopathic effect in primary mouse embryo monolayers that had been infected with wild type polyoma. The cell line has now been releasing virus in culture for more than

1 year, and the plaques made by the virus are still of the mutant type. The mutant has been designated as *sp* (= small plaque). Cross-neutralization of plaques has so far shown no antigenic difference between *sp* and the wild type.

sp virus harvested 8 months after the establishment of the cell line was tested for tumour induction *in vivo*. In contrast to a 100 per cent incidence of animals with tumours after inoculation of wild type polyoma into hamsters, even with an input of 1·0–3·0 × 10^5 plaque-forming units of *sp* per animal, only subcutaneous sarcomas were found in 3 out of 31 hamsters (Table 1). One litter of *AKR* mice was inoculated when 2 days old with *sp*, and during an observation period of 6 months, parotid tumours only were observed in 1 out of 5 animals, in contrast to a 100 per cent tumour induction after inoculation of wild type virus.

Table 1. Tumour Induction in Hamsters after Inoculation with Wild Type Polyoma Virus and the Mutant *sp*

Virus	No. of litters	Age at inoculation (days)	Input virus per animal (plaque-forming units)	Observation period (months)	No. with tumour / No. of animals
Wild type	6	3 to 6	1·0 × 10^5	2·5	30/30
sp	3	2 to 4	1·0 to 3·0 × 10^5	2·5	1/16
	3	3 to 6	1·4 to 2·0 × 10^5	3 to 4	2/15

Animals inoculated subcutaneously with 0·1 ml. of virus suspension.

These results show that it is possible to obtain, with a tumour virus, a mutant that is 'attenuated' regarding its tumour-producing capacity, while retaining its cytopathic effect. The existence of complete attenuation for tumour-producing capacity, and of the reverse type of mutant, that is, tumour production without cytopathic effect, is now being looked for. It is of interest that in common with the *d* [4] and *MS* [5] mutations of polyomyelitis that can be associated with reduced neuropathogenicity, the reduced tumour induction by this polyoma mutant is also associated with a plaque type mutation.

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Virus and Antibodies in the Lymph and Blood of Sheep infected experimentally with the Virus of Tick-borne Encephalitis

It is known that foreign substances which penetrate the organism are resorbed by the lymphatic system. This also applies to bacteria and viruses.

In previous experiments[1] it was found that when tick-borne encephalitis virus was administered subcutaneously to mice, it was resorbed via the regional lymph vessels into the blood stream, from which it afterwards penetrated further lymph vessels and other organs. This was demonstrated by detecting the virus in the lymph nodes and blood.

Since I was interested in the question of the part played by the lymphatic and blood vascular systems in direct resorption of the virus from infected tissue, in their time relationships and in the antibody response in the lymphatic system, it was necessary to increase the accuracy of the preceding experiments by examining more adequate systems, namely, lymph and blood.

The experiments were carried out in sheep with an artificial lymph-blood circulation[2]. Lymph was collected from the cannulated thoracic duct and blood was obtained from a contralateral vein (one of the branches of the right jugular vein). The virus was detected in mice by intracerebral infection. Neutralizing antibodies were determined by an intraperitoneal test in mice.

The experiments clearly demonstrated that the lymphatic system is the main route by which the virus penetrates the blood stream from the subcutaneous tissue. No direct resorption of the virus from the subcutaneous tissue into the blood stream was found. If this occurs at all, it is evidently on such a small scale that it cannot be detected by the method used. The rate of resorption by the lymphatic system depends on the amount of virus. When administered subcutaneously into the metatarsus of the hind limb in amounts of 0·043 ml. 20 per cent suspension/1 kgm. body-weight (about three to four thousand million intracerebral mouse *LD*50), the virus was detected 60 min. later in lymph collected from the thoracic duct. No penetration of the virus into the blood stream from any part of the lymphatic system other than the thoracic duct was observed. Following re-infection seven and twelve days after primary inoculation, the virus was not detected in the lymph or the blood, even at very early intervals after reinfection[3].

Neutralizing antibodies were detected in the lymph sooner and in higher titres than in the blood plasma. Antibodies were also determined in the lymph of a sheep in which the thoracic duct was exteriorized and its connexion with the blood stream was interrupted, so that the virus, which was administered into a peripheral lymph vessel, passed through the regional lymph vessels only. This means that antibodies were determined in the lymph after antigenic stimulation to which, in effect, only the regional lymph vessels were exposed. The first antibodies were always found when the virus rapidly decreased in the lymph and in the blood. The higher antibody titre in the lymph was maintained up to the seventh day after infection. From then onwards it was higher in the blood plasma. Re-infection on the seventh and twelfth day after primary inoculation resulted in a further antibody increase in the lymph and the blood plasma, which was greater in the latter[4].

These experiments show that the lymphatic system plays an important part in resorption and transport of the tick-borne encephalitis virus and in the formation of antibodies against it.

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[2] Málek, P., and Kolc, J., *Casopis lékaru ceskych*, **34**, 1069 (1958).
[3] Málková, D., *Acta Virologica* (in the press).
[4] Málková, D., *Acta Virologica* (in the press).

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

BRANCH PRINCIPAL (with a good degree and suitable experience in universities or technical colleges) OF THE ENUGU BRANCH of the Nigerian College of Arts, Science and Technology—The Council for Overseas Colleges, 12 Lincoln's Inn Fields, London, W.C.2 (August 26).

LECTURERS (with an honours degree, or equivalent, in engineering or applied science, and industrial, research or teaching experience) IN MECHANICAL ENGINEERING—The Registrar, Royal Technical College, Salford, Lancs (August 31).

RESEARCH FELLOW (microbiologist with postgraduate research experience, and preferably experience in microbial genetics) IN MICROBIOLOGY, to join a research team of chemists and microbiologists concerned with fundamental studies on biosynthesis—The Registrar, University College, Singleton Park, Swansea (August 31).

PHYSIOLOGISTS (ASSISTANT LECTURERS, LECTURERS, or SENIOR LECTURERS) (with a degree in physiology, of honours standard, and for one of the appointments preference will be given to a candidate with postgraduate training and research experience in the field of human physiology) IN THE NEW DEPARTMENT OF ERGONOMICS AND CYBERNETICS, for research and postgraduate teaching in ergonomics—The Academic Registrar, Loughborough College of Technology, Loughborough, Leicestershire, quoting Ref. 17/AF (September 1).

RESEARCH ASSISTANT (preferably with research experience) IN THE DEPARTMENT OF BOTANY, for work in translocation in plants—The Secretary, The University, Aberdeen (September 3).

SENIOR LECTURER or LECTURER (with special qualifications either in plant pathology or virology) IN BOTANY—Joint Clerk to the University Court, Queen's College (University of St. Andrews), Dundee (September 3).

ASSISTANT DIRECTOR OF RESEARCH IN DYNAMICAL OCEANOGRAPHY in the Department of Applied Mathematics and Theoretical Physics—Dr. F. G. Friedlander, Secretary of the Appointments Committee of the Faculty of Mathematics, the Arts School, the University, Bene't Street, Cambridge (September 9).

ASSISTANT LECTURER (graduate, with special interests in either igneous petrology or sedimentary petrology and palæozoic stratigraphy) IN GEOLOGY—The Registrar, The University, Leicester (September 10).

LECTURER (with a good honours degree in electrical engineering or physics, preferably of a British university, together with some industrial experience, and preferably some teaching experience and a special knowledge of microwave engineering) IN ELECTRICAL ENGINEERING—The Secretary, University College, Gower Street, London, W.C.1 (September 10).

ASSISTANT EXPERIMENTAL OFFICER (with a pass degree or five subjects at G.C.E. "O" level and at least two at "A" level) IN THE DEPARTMENT OF POTATO GENETICS, to assist in routine tests and pathological investigations—The Secretary, John Innes Institute, Bayfordbury, Hertford, Herts (September 12).

SENIOR LECTURER IN HORTICULTURE at the University of Sydney, Australia, to undertake teaching and research in fruit and vegetable production—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, September 13).

EXPERIMENTAL OFFICER (MICROBIOLOGIST) (with experience in the isolation and maintenance of pure bacterial cultures, and preferably experience in the biochemical techniques) at the National Chemical Laboratory, D.S.I.R., Teddington, Middlesex, to join a team studying the influence of micro-organisms on corrosion of metals, particularly in soil—The Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting G.275/0A (September 19).

BOTANIST or BIOCHEMIST (with an interest in field work), to grow and test plants for suitability as sources of protein—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (September 20).

LECTURER IN ANATOMY at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, September 30).

SENIOR LECTURER IN PHYSICS, and LECTURERS (2) IN PHYSICS at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, September 30).

PHYSICIST or CHEMIST, Scientific Officer grade (male, with a first- or second-class honours degree, and preferably experience of polymers or biological systems or ionizing radiation), at the Royal Military College of Science, Shrivenham, for a two- or three-year programme of fundamental research (of an academic character and intended for publication) on effects of high energy radiation of polymers in solutions, and the effect of additives as a means of radiation protection or enhancement. This work would be correlated with corresponding effects in biological systems—Technical and Scientific Register (K), Ministry of Labour, 26 King Street, London, S.W.1, quoting A.326/0A (October 6).

AGRICULTURAL OFFICER (EDUCATION) (male, with a degree in agriculture or natural science, at least two years suitable postgraduate experience or training, and preferably some teaching experience) in Nyasaland, to teach agricultural subjects to African students at the Colby School of Agriculture near Lilongwe—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/4/023/T.

ASSISTANT LECTURER (Grade B) IN MATHEMATICS—The Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S.W.3.

ASSISTANT (with a degree in botany or agriculture, and preferably experience of plant pathology) TO THE MYCOLOGIST—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

BIOLOGIST (with a good degree in physiology or zoology and considerable research experience preferably with spermatozoa), to join a small research team investigating cervical mucus, with particular reference to problems of fertility—The Secretary, National Institute for Research in Dairying, Shinfield, Reading, quoting Ref. 60/13.

CHEMISTS, MATHEMATICIANS (including STATISTICIANS), METALLURGISTS, MECHANICAL AND ELECTRICAL ENGINEERS, and PHYSICISTS (including BIOPHYSICISTS) (men and women, with a first- or second-class honours degree or equivalent) at Research and Development Establishments of the War Office at various locations, including Sevenoaks, Kent; Byfleet, Chertsey and Tolworth, Surrey; Shoeburyness, Essex; Christchurch and Farnborough, Hants; Salisbury, Wilts; and in Cornwall—Technical and Scientific Register (K), Ministry of Labour, 26 King Street, London, S.W.1, quoting A.300/0A.

DEMONSTRATOR IN AGRICULTURAL ZOOLOGY at the School of Agriculture, Sutton Bonington—The Registrar, The University, Nottingham.

ELECTRONICS TECHNICIAN (male or female), for research work—Dr. E. Bülbring, Department of Pharmacology, The University, South Parks Road, Oxford.

EXPERIMENTAL OFFICER or ASSISTANT EXPERIMENTAL OFFICER (preferably with a degree in dairying, or the N.D.D., together with good experience of laboratory work) IN THE BACTERIOLOGY DEPARTMENT, to work on hygienic aspects of milk production and processing—The Secretary, National Institute for Research in Dairying, Shinfield, Reading, quoting Ref. 60/8.

EXPERIMENTALIST (with G.C.E. at "A" level, H.N.C. in applied physics or equivalent qualifications and preferably an interest in electronics) IN THE METALLURGY DIVISION, A.E.R.E., to assist with experimental measurements for the study of solids by diffracting beams of neutrons from nuclear reactors—The Personnel Manager (1747/34), United Kingdom Atomic Energy Authority, Atomic Energy Research Establishment, Harwell, Didcot, Berks.

GEOLOGISTS (not over 35, with a first- or good second-class honours degree in geology) with the Government of Northern Nigeria, for regional geological mapping and the examination of mineral deposits—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.105/3/01.

HONOURS GRADUATE (preferably in physics), for work on electrical and mechanical properties of ceramics at ordinary and elevated temperatures—The Director of Research, British Ceramic Research Association, Queens Road, Penkhull, Stoke-on-Trent.

LABORATORY TECHNICIAN (with some training in bacteriological technique and preferably with Inter. I.M.L.T.)—The Secretary, Royal Veterinary College, Royal College Street, London, N.W.1.

MALE SPECIALIST VIROLOGIST (with qualifications registrable in the United Kingdom, and a minimum of seven years postgraduate experience, at least five of which must have been spent in the practice of virology) in Hong Kong, to take charge of the Vaccine Institute and the manufacture of human and animal vaccines—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD117/51/062.

PHYSICAL CHEMIST (with at least five years research experience) IN THE RESEARCH LABORATORIES, initially to lead a team studying the bulk properties of high polymers—The Director, Arthur D. Little Research Institute, Inveresk, Midlothian, Scotland.

PHYSICAL CHEMIST (with doctorate, preferably with experience in low temperature calorimetry), for academic research in the thermodynamic properties of compounds of biochemical interest—Institute of Scientific Research, New Mexico Highlands University, Las Vegas, New Mexico, U.S.A.

PLANT PHYSIOLOGIST (with a good honours degree in botany or chemistry with specialization in plant physiology and not less than two years suitable postgraduate experience or training) in Tanganyika, for research work on coffee, with particular reference to over bearing and plant nutrition, and to collaborate with other specialists—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/8/026/T.

POST-DOCTORAL FELLOWS IN THE DEPARTMENT OF CHEMISTRY, for research work in the fields of electron spin resonance spectroscopy of free radicals at liquid helium temperature, and mass spectroscopy including photo-ionization studies—Prof. C. A. McDowell, Department of Chemistry, University of British Columbia, Vancouver 8, B.C., Canada.

POST-DOCTORAL RESEARCH FELLOW IN THE DEPARTMENT OF ORGANIC CHEMISTRY, for work on biosynthesis in fungi and bacteria—The Registrar, The University, Liverpool, quoting Ref. CV/N.

RESEARCH ASSISTANT (PHYSICIST) (with a knowledge of electronics and a degree in physics), for work with the X-ray scanning microanalyser (electron probe) in the field of ore microscopy—Dr. A. P. Millman, Mining Geology Department, Royal School of Mines, Prince Consort Road, London, S.W.7.

SCIENTIFIC ASSISTANT (ABSTRACTOR) (with a degree in agriculture or natural science and a knowledge of languages)—The Director, Commonwealth Bureau of Pastures and Field Crops, Hurley, Berkshire.

SENIOR LECTURER IN SERVOMECHANISMS AND CONTROL ENGINEERING, a SENIOR LECTURER IN STRUCTURAL ENGINEERING, a SENIOR LECTURER IN ENGINEERING DRAWING, a LECTURER IN THE STRENGTH OF MATERIALS AND ELASTICITY, a LECTURER and an ASSISTANT LECTURER IN APPLIED MECHANICS, a LECTURER IN ENGINEERING DRAWING, a LECTURER IN THERMODYNAMICS, a LECTURER IN PRODUCTION ENGINEERING, and a LECTURER IN CHEMICAL ENGINEERING, IN THE DEPARTMENT OF MECHANICAL, CIVIL AND CHEMICAL ENGINEERING—The Secretary, Royal College of Science and Technology, George Street, Glasgow, C.1.

SENIOR LECTURER (with good teaching experience, together with research or industrial experience) IN INORGANIC or IN PHYSICAL CHEMISTRY—The Principal, Huddersfield College of Technology, Huddersfield.

SENIOR SCIENTIFIC GLASSBLOWER (well qualified, with considerable experience in high vacuum techniques, metal/glass seals, quartz work, etc.) IN THE DEPARTMENT OF CHEMISTRY—The Secretary, Department of Chemistry, University of British Columbia, Vancouver, B.C., Canada.

SENIOR TECHNICIAN II, to take charge of the Biochemistry Department in the Group Laboratory, Connaught Hospital, Walthamstow—The Secretary, Forest Group H.M.C., Langthorne Road, London, E.11.

SPECIALIST OFFICER (PLANT PATHOLOGIST) (with a university degree in natural science (botany) with first- or second-class honours,

and two years postgraduate experience in plant pathology) IN THE DEPARTMENT OF AGRICULTURAL RESEARCH, Ibadan, Nigeria, to undertake research into virus diseases of food crops—The Appointments Secretary, Federal Public Service Commission, Nigeria House, 9 Northumberland Avenue, London, W.C.2, quoting B.7/2.

TECHNICIAN (with some knowledge of, and interest in, insects or spiders)—Prof. G. C. Varley, Hope Department, University Museum, Oxford.

VISITING ASSISTANT PROFESSOR-RESEARCH ASSOCIATE, to give some lectures in general chemistry and in an advanced course in inorganic chemistry, help with the supervision of laboratory work, and engage in research in inorganic chemical synthesis or reactions—Louis J. Bircher, Box 1550, Vanderbilt University, Nashville 5, Tennessee, U.S.A.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Department of Scientific and Industrial Research: Road Research Laboratory. Road Note No. 25: Sources of White and Coloured Aggregates in Great Britain (1959). Pp. 15. (London: H.M. Stationery Office, 1960.) 1*s.* 3*d.* net. [136

Commonwealth Mycological Institute, Kew. Mycological Papers. No. 76: Dematiaceous Hyphomycetes: 1. By M. B. Ellis. Pp. 36. No. 77: A Supplement to a Host List of Plant Diseases in Malaya. By A. Johnston. Pp. 30. 7*s.* 6*d.* net. (Kew: Commonwealth Mycological Institute, 1960.) [136

British Broadcasting Corporation. Television Field Trials of 405-line and 625-Line Systems in the UHF and VHF Bands, 1957–1958. Part 1: Results of Field Trials of 405-Line Television in Bands I and V. Part 2: Results of Field Trials of 405-Line Television in Band I and 625-Line Television in Band V. Pp. 152. (London: British Broadcasting Corporation, 1960.) 20*s.* [136

Flora of Tropical East Africa. Edited by C. E. Hubbard and E. Milne-Redhead. Rosaceae. By R. A. Graham. Pp. 61. (London: Crown Agents for Oversea Governments and Administrations, 1960.) 5*s.* [136

Lawes Agricultural Trust. Report of the Rothamsted Experimental Station for 1959. Pp. 288. (Harpenden: Rothamsted Experimental Station, 1960.) 10*s.* [136

British Society for the Promotion of Vegetable Research. National Vegetable Research Station Tenth Annual Report 1959 (Oct. 1958–Sept. 1959). Pp. viii+63. (Wellesbourne, Warwick: National Vegetable Research Station, 1960.) [136

Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 85: The Propagation of Trees. Pp. 12. (Belfast: Ministry of Agriculture, 1960.) [136

Scientific Proceedings of the Royal Dublin Society. Series A. Vol. 1, No. 2: Light Collection on Liquid Scintillator Cells. By D. O. Cummins, C. F. G. Delaney and I. R. McAulay. Pp. 21–26. 1*s.* Vol. 1, No. 3: The Stratigraphy and Structure of the Lower Palaeozoic Rocks of North-West Galway. By Dr. W. S. McKerrow and Dr. C. J. Campbell. Pp. 27–52+plates 1–6. 8*s.* Series B. Vol. 1, No. 1: An Investigation of Winter Dormancy in *Heterodera rostochiensis*. By P. C. Cunningham. Pp. 1–4. 1*s.* Vol. 1, No. 2: Problems of Grass Conservation in Ireland. By J. R. O'Callaghan. Pp. 5–20. 4*s.* (Dublin: The Royal Dublin Society, 1960.) [136

John Innes Horticultural Institution. Fiftieth Annual Report, 1959. Pp. 50. (Bayfordbury, Hertford: John Innes Horticultural Institution, 1960.) 3*s.* [136

The Carnegie United Kingdom Trust. 46th Annual Report, 1959. Pp. viii+68. (Dunfermline, Fife: Carnegie United Kingdom Trust, 1960.) [136

Department of Agriculture and Fisheries for Scotland. Spaced-Plant Trials at East Craigs. 6: Varieties of Perennial Ryegrass, *Lolium perenne* L. 1956–58. Pp. 7+2 figures. (Edinburgh: Department of Agriculture and Fisheries for Scotland, 1960.) [136

Department of Scientific and Industrial Research: Building Research Station. Factory Building Studies No. 5: Sites and Foundations. Pp. iv+20. (London: H.M. Stationery Office, 1960.) 3*s.* net. [136

Department of Scientific and Industrial Research: National Engineering Laboratory. Mechanical Engineering Research 1959: Report of the National Engineering Laboratory Steering Committee, with the Report of the Director of the National Engineering Laboratory. Pp. iii+64+4 plates. (London: H.M. Stationery Office, 1960.) 4*s.* 6*d.* net. [146

British Film Institute. National Film Archive Catalogue, Part 2: Silent Non-Fiction Films, 1895–1934. Pp. x+195+12 plates. 15*s.* The National Film Archive. Pp. 16. 2*s.* 6*d.* (London: British Film Institute, 1960.) [146

Ministry of Power. Reports of H.M. Inspectors of Mines and Quarries under the Mines and Quarries Act, 1954, for 1959. Durham Division. By W. Widdas. Pp. iv+39+5 plates. 3*s.* 6*d.* net. East Midland Division. By H. S. Stephenson. Pp. iv+41+5 plates. 3*s.* 6*d.* net. (London: H.M. Stationery Office, 1960.) [146

Royal Greenwich Observatory Bulletins. No. 19: Time Service 1959, July–September. Pp. 259–276. (London: H.M. Stationery Office, 1960.) 3*s.* 6*d.* net. [146

Department of Scientific and Industrial Research: Building Research Station. Tropical Building Studies No. 1: Densities in Housing Areas. By P. H. M. Stevens. Pp. 55. (London: H.M. Stationery Office, 1960.) 10*s.* net. [146

Educational Productions Limited. Filmstrip No. C6348: The Hedgerow. 36 frames. 27*s.* 6*d.* Notes for use with Filmstrip No. C6348. Pp. 12. Filmstrip No. C6349: Beautiful Butterflies. 31 frames. 27*s.* 6*d.* Notes for use with Filmstrip No. C6349. Pp. 16. Filmstrip No. C6403: Flowers of Marsh and Pool. 31 frames. 27*s.* 6*d.* Notes for use with Filmstrip No. C6403. Pp. 13. Filmstrip No. C6430: The Woodlands. 35 frames. 27*s.* 6*d.* Notes for use with Filmstrip No. C.6430. Pp. 13. (East Ardsley, Wakefield: Educational Productions, Ltd., 1959 and 1960.) [156

Other Countries

Proceedings of the California Academy of Science. Fourth Series. Vol. 29, No. 16: The Morphology of *Phyllapiyxis zostericola*, New Species. By James E. McCauley. Pp. 549–576. Vol. 29, No. 17: Rhoicinine Spiders (Pisauridae) of Western South America. By Harriet Exline. Pp. 577–620 (5 plates). (San Francisco: California Academy of Sciences, 1960.) [315

Metropolitan Life Insurance Company. Statistical Bulletin, Vol. 41 (April 1960): Overweights Benefit from Weight Reduction. Why Older People are Hospitalized. The American Family Today. Loss of Life in Violent Storms and Floods. Pp. 12. (New York: Metropolitan Life Insurance Company, 1960.) [315

Geological Survey of Tanganyika. Bulletin No. 30: Microfloral Investigation of the Lower Coal Measures (K2); Ketewaka-Mchuchuma Coalfield, Tanganyika. By George F. Hart. Pp. iii+18+3 plates. Shs. 5. Memoir No. 1: Summary of the Geology of Tanganyika. Part 2: Geological Map. Pp. 6+map. Shs. 5. (Dar-es-Salaam: Government Printer, 1960.) [315

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United States Department of Agriculture: Agricultural Research Service. Technical Bulletin No. 1222: Collecting Insects by Airplane, with special reference to Dispersal of the Potato Leafhopper. By Perry A. Glick. Pp. ii+16. (Washington, D.C.: Government Printing Office, 1960.) 10 cents. [315

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Universidade de Sao Paulo. Contribuicoes Avulsas do Instituto Oceanografico. No. 2: Os Equinodermas do Litoral de Sao Paulo, II. Por Luiz Roberto Tommasi. Pp. 27+6 plates. No. 3: Sôbre duas Arenicolas da Costa Brasileira (Annelida, Polychaeta). Por E. Nonato. Pp. 6+1 plate. Catalogie of Marine Larvae. By M. Vannucci. Pp. 44+74 figures. (Sao Paulo: Universidade de Sao Paulo, Instituto Oceanografico, 1958 and 1959.) [315

Annals of the New York Academy of Sciences. Vol. 84, Article 4: The Organization of Psychiatric Care and Psychiatric Research in the Union of Soviet Socialist Republics. By Nathan S. Kline. Pp. 147–224. Vol. 84, Article 5: A New Method for Cytological Diagnosis of Pulmonary Cancer. By Ludwig von Bertalanffy and Felix F. Bertalanffy. Pp. 225–238. Vol. 84, Article 6: The Kinetics of Reactions at Changing Temperature. By C. Olin Ball. Pp. 239–250. Vol. 85, Article 2: Freezing and Drying of Biological Materials. By Harold T. Merrymas and 12 other authors. Pp. 501–734. (New York: New York Academy of Sciences, 1960.) [315

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World Health Organization. Technical Report Series. No. 194: Local Health Service—Third Report of the Expert Committee on Public Health Administration. Pp. 49. (Geneva: World Health Organization; London: H.M. Stationery Office, 1960.) 2 Swiss francs; 3*s.* 6*d.*; 0.60 dollars. [315

U.S. Department of Health, Education, and Welfare. Public Health Service Publication No. 735: Highlights of Progress in Mental Health Research 1959: Items of Interest on Program Developments and Research Studies conducted and supported by the National Institute of Mental Health. Pp. v+51. (Washington, D.C.: Government Printing Office, 1960.) 25 cents. [315

U.S. Department of the Interior: Fish and Wildlife Service. Fishery Bulletin No. 169: Definition of Haddock Stocks of the Northwestern Atlantic. By John R. Clark and V. D. Vladykov. Pp. iv+283–296. 20 cents. Fishery Bulletin 170; Annotated Bibliography on Biology of American Menhaden. By John W. Reintjes, James Y. Christman, Jr., and Richard A. Collins. Pp. iv+297–322. 25 cents. (Washington, D.C.: Government Printing Office, 1960.) [315

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Canada Department of Agriculture. Annual Report of the Forest Insect and Disease Survey, 1959. Pp. 121. (Ottawa: Queen's Printer, 1960. Obtainable from Information Division, Canada Department of Agriculture, Ottawa.) [36

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